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ALLERGY TO PYRAZOLONE DERIVATIVES (AMINOPYRINE) WITH EVIDENCE OF A REAGINIC TYPE ANTIBODY

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WE ARE reporting here a case of allergy to pyrazolone derivatives, mainly aminopyrine. The interest of this phenomenon lies in its clinical and immunologic features.

The patient presents, after ingestion of aminopyrine, symptoms of an anaphylactic-like shock. Skin tests with aminopyrine are strongly positive. Passive transfer to a nonallergic person has been regularly successful and strongly positive.

Investigations on the physical and immunologic properties of the antibody involved in this drug allergy have been made and will be reported here.

CASE REPORT

Patient A. B., a 46-year-old man, was a French literature professor. His physician sent him to our outpatient department because of sudden loss of consciousness which occurred after the appliance of a suppository of Optalidon. The diagnosis suggested at the time by his family physician was "epileptic seizure."

The patient's family history was of little significance except for the facts that his father died of lung carcinoma and that his mother had bronchial asthma and eczema. The patient was married and the father of two children—a 10-year-old boy and a 20-year-old daughter. The daughter had bronchial asthma due to feathers.

The patient had measles and mumps in childhood; in addition, the history was marked with numerous allergic accidents, which will be described.

At the age of 16 he developed a contact allergy to his spectacle frames (probably made of celluloid). These were then replaced by gold frames.

The first symptoms of drug allergy appeared in 1931, when he was 20 years old. With his breakfast one morning he took a "composite" tablet for a severe headache. A few minutes later, while walking in the street, he felt a violent itching of the scalp, face, tongue, and palms. Very rapidly his face and the upper part of his body were covered with a giant urticaria. His tongue swelled rapidly and he felt some shortness of breath. He vomited his breakfast and fell unconscious in the street. When he awakened, he was in his bed. He can remember that the physician, after injecting Adrenalin, instructed him always to keep a vial of Adrenalin drops handy.

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In the years that followed, at intervals which the patient could not recall exactly, he was bothered by the same onset of symptoms, but immediate perlingual absorption of Adrenalin stopped their further development.

In 1945 there was a similar accident, following absorption of an anti-influenza tablet, characterized by the same symptoms, namely, violent itching, edema of the face and tongue, vomiting, and loss of consciousness. That time Adrenalin drops failed to prevent the troubles.

From 1945 to 1956 the patient avoided all analgesics but, meanwhile, he experienced contact dermatitis to plastics. Skin tests performed at the time confirmed a sensitivity to vinyl products.

In December, 1956, he suffered an attack of severe influenza with high temperature, arthralgia, myalgia, and headache. His physician prescribed suppositories of Optalidon* and benzyl penicillin tablets. Shortly after the introduction of the suppository, he presented the same typical symptoms followed by loss of consciousness. The physician, called back in that emergency, gave him an injection of Phenergan. This last event led to our consultation in the Outpatient Department of the Hospital Broussais. The patient presented himself with a letter from the physician who prescribed the drug and who was called back when the patient collapsed. The diagnosis was "epileptic seizure."

Physical Examination.—The patient was well developed. His pulse rate was 62 and his blood pressure was 125/75 mm. Hg. He was 171 cm. tall and weighed 67 kg. The physical examination, including careful respiratory, cardiovascular and neurological evaluation, was normal except for evidence of dermatitis around the neck and wrists and on the scrotum.

Fluoroscopy showed a slight degree of emphysema.

Laboratory Data.—The laboratory findings on Dec. 14, 1956, were as follows:

Urinalysis: no sugar, no albumin.
Red blood cell count: 5,200,000 with 105 per cent hemoglobin.
White blood cell count: 7,250.
Platelets: 330,000.
Bleeding time: 2 minutes and 30 seconds.
Coagulation time: 8 minutes and 30 seconds.

White cell differential:

Segmenters—	
Neutrophils	64 per cent
Eosinophils	1 per cent
Basophils	0 per cent
Lymphocytes—	
Small	14 per cent
Large	11 per cent
Monocytes	10 per cent

Sedimentation rate (Westergren): First hour, 3 mm.; second hour, 10 mm.; third hour, 17 mm.; fourth hour, 27 mm.; twenty-fourth hour, 70 mm.

A resorcin flocculation test (optical density of serum) was normal. The localization of skin dermatitis was obviously related to the contact of the patient's raincoat, which was made of vinyl chloride material. Therefore, we made some patch tests with various plastics and with rubber, penicillin, and sulfanilamides, with the following results:

Vinyl plastics	++
Rubber	+
Penicillin	–
Nylon (stockings)	–
Sulfanilamide	–
Celluloid	++

**Optalidon suppository:* Isobutylallyl-barbital 0.15 Gm., 1-phenyl-2-3 dimethyl-4 dimethylamino pyrazolone 0.375 Gm., caffeine 0.075 Gm.

As the patient's physician stated in his letter that the attack at which he assisted was an epileptic seizure, we decided to ask for an electroencephalogram. The picture was perfectly normal; therefore, the patient was persuaded to absorb two tablets of aminopyrine (0.5 gr. each), while the electroencephalogram was recorded. Dr. François Lhermitte, Associate Professor of Neurology, kindly agreed to perform this unusual examination. Eight minutes after absorption of the drug the patient began to complain of swelling of the scalp, face, and palms. Urticarial blotches rapidly covered his face. Two minutes later he felt extremely uncomfortable. He was pale and covered with sweat, he vomited, and rapidly lost consciousness. No convulsion occurred; the pulse was impalpable and arterial blood pressure was so low that no measure was practicable. The electroencephalogram, which remained normal until the patient's collapse, suddenly changed and showed slow waves of the delta type. No clinical signs of epilepsy were noted. The picture was thus one of collapse.

The patient then received injections of Adrenalin and of Phenergan. In a few minutes he recovered and two hours later he was able to leave the clinic.

Thus, we became convinced that the troubles suffered by this patient after absorption of aminopyrine were symptoms of anaphylactic shock.

1. *Skin Test.*—In scratch tests performed on this patient, several drugs were tested. Table I shows the results which we observed about fifteen minutes after the application of the various substances.

TABLE I

Aminopyrine	+++
Optalidon	+++
Antipyrine	+
Iodoantipyrine	++
Phenacetin	?
Aspirin	–
Butazolidin	–
Potassium iodide	–
Control	–

With aminopyrine and Optalidon (containing aminopyrine) the sites of application rapidly became red and pruriginous. After fifteen minutes there was a typical wheal-and-flare reaction around the scratches, with pseudopodial prolongments (Fig. 1). The tests were repeated three times, at ten-day intervals, and the same results were noted.

2. *Passive Transfer in Human Beings.*—A blood sample was taken under sterile conditions, and the serum obtained was divided into two parts. The first portion was lyophilized at a low temperature; the second was stored in the freezer after adjunction of Merthiolate at 1:10,000 solution.

Passive transfer tests were performed with the normal serum, as well as with the lyophilized serum, on fifteen persons. The reaction was strongly positive in twelve (Fig. 2), slightly positive in one, and doubtful in two. Results were identical with both samples.

(a) *Oral absorption of the challenging drug (aminopyrine):*

Miss Mon. Intradermal injections of 0.1 ml. of stored serum of Patient A. B. and 0.1 ml. of a control nonallergic serum (B. N. H.) were made in the skin of the forearm.

Twenty-four hours later Miss Mon. swallowed a tablet of aminopyrine (0.5 Gm.). Ten to fifteen minutes after ingestion the site where Patient A. B.'s serum had been injected was swollen, pruriginous, and intensely red.

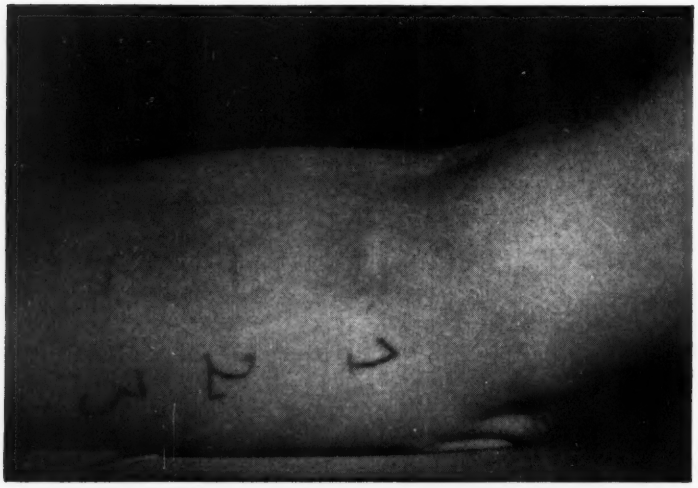


Fig. 1.—Patient A. B. Scratch tests performed with: *1*, aminopyrine (+++), *2*, aspirin (−), and *3*, phenacetin (−).

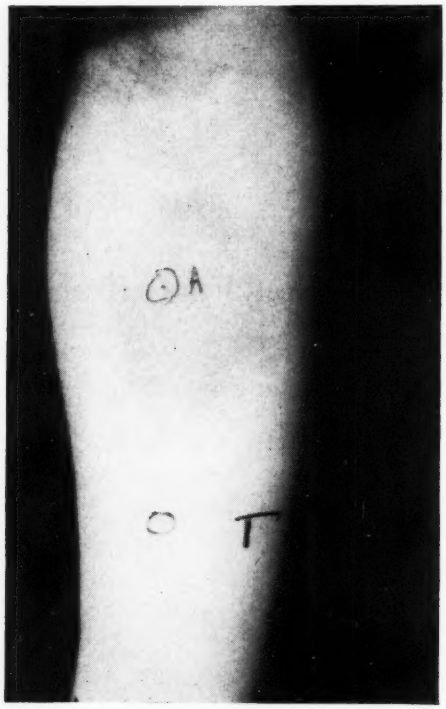


Fig. 2.—Passive transfer. The serum of Patient A. B. has been injected intradermally into the arm of Miss Mon. at *A*, while a control serum has been injected at *T*. Twenty-four hours later, a tablet of aminopyrine has been absorbed by Miss Mon. The reaction was positive (++++) at the site of injection of the patient's serum and negative at the site of injection of the control serum.

After thirty minutes there was a red, edematous reaction, 5 to 6 cm. in size. The reaction was qualified ++++. The site of injection of the control serum (B. N. H.) showed no modification (Fig. 2).

Miss Ther. Intradermal injections of 0.1 ml. of lyophilized serum of Patient A. B. diluted in saline (0.9:100) and 0.1 ml. of a control serum (B. N. H.) were made in the skin of the forearm. Twenty-four hours later ingestion of a tablet of aminopyrine (0.5 Gm.) induced, in thirty minutes, a strong reaction on the skin at the site of the injection of Patient A. B.'s serum and none at the site of the control serum (B. N. H.). The skin was intensely red, swollen, and pruriginous. The reaction was qualified ++++.

Mr. T. Z. A ward patient, with no history of allergy, received intradermal injections of Patient A. B.'s serum, 0.2 ml. in the left forearm, and control serum (B. N. H.), 0.2 ml. in the right forearm. Twenty-four hours later he ingested two tablets of aminopyrine (1 Gm.). In the next five to six minutes the skin began to be red, swollen, and pruriginous at the site of the injection of the patient's serum. After thirty minutes this site was intensely swollen, red, and hot. The reaction was qualified +++++.

We were therefore able to realize a successful passive transfer of antibody with a very strong positive reaction after oral absorption of the antigen (aminopyrine).

(b) *Intradermal injection of the challenging drug:* The same procedure was applied in three other subjects, the only difference being that, instead of being ingested, aminopyrine was administered intradermally (0.2 ml. in a 2:100 solution) at the sites where the serum was injected. In none of the three subjects was a positive reaction noticed. The total failure to obtain a positive response after the intradermal injection of the drug antigen at the site where the antibody was previously injected, in contrast with the extremely positive reaction after ingestion of the drug, has been a surprise to us. We shall discuss later the various eventualities suggested by this discrepancy.

(c) *Intramuscular injection of the aminopyrine:*

Mr. d'Acro. Patient A. B.'s serum and control serum (B. N. H.) were injected simultaneously in the skin of the right forearm of our subject. The following day we injected 5 ml. of a 5:100 dilution of aminopyrine (0.25 Gm.) intramuscularly. Twenty minutes later the site of injection of the serum of Patient A. B. was red and swollen. The reaction was qualified ++++. The reaction to the control serum remained negative.

While intradermal injection of aminopyrine in the site of the injection of the immune serum was unsuccessful, strong positive reactions are obtained regularly by oral absorption or parenteral injection of aminopyrine.

3. *Passive Transfer in Guinea Pigs.*—Attempts at passive transfer in guinea pigs have been made by the technique described by Ovary.[14] The immune serum has been injected intradermally in several dilutions. Six hours later 0.5 ml. of a 1:100 solution of aminopyrine containing Evans blue dye has been administered intravenously. In none of the six experiments was there evidence of a local antigen-antibody reaction.

4. *The Local Injection of Aminopyrine Does Not Block the Specific Antibody.*—An experiment was made on Mr. N. Y., a ward patient. On December 12, he was given an intradermal injection of 0.1 ml. of Patient A. B.'s serum in four

different sites on the back, which we shall designate *a, b, c,* and *d,* and an injection of a control serum (*e*).

On December 15 he was given the following:

Injection of 0.2 ml. of aminopyrine (2:100 dilution) at *a.*
Injection of 0.2 ml. of a saturated solution of aspirin at *b.*
Injection of 0.2 ml. of a saturated and filtrated solution of Optalidon at *c.*
No drug injection at site *d.*
Injection of 0.2 ml. of a solution of aminopyrine 2:100 at the site of injection of the control serum (*e*).

Twenty minutes later all the skin reactions were negative. One hour later, Mr. N. Y. took 1 Gm. of aminopyrine orally. After twenty minutes reactions were strongly positive and equal in intensity at sites *a, b, c,* and *d.* Reactions were qualified ++. The control serum (*e*) was negative.

5. *Rapidity of Fixation of the Antibody on the Tissue Cells.*—In order to determine the time required for fixation of the antibody to the tissue cell, aminopyrine was given at different intervals after the intradermal injection of 0.1 ml. of the serum of Patient A. B. The results are shown in Table II.

TABLE II

TIME OF INGESTION	RESULTS
Simultaneously	−
2 hours	±
4 hours	++++
6 hours	++++
12 hours	++++
24 hours	++++

It should be emphasized that the fixation of antibody in human skin is relatively rapid, since the fixation already seems to be maximal four hours after the intradermal injection.

6. *Attempts to Evaluate the Quantity of Antibody by Successive Dilutions of the Patient's (A. B.) Serum.*—In order to have an approximate idea of the antibody titer of the serum of patient A. B., intradermal injections of 0.1 ml. of successive dilutions in saline were made.

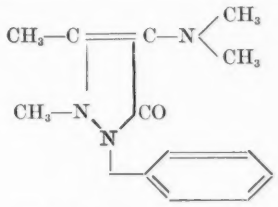
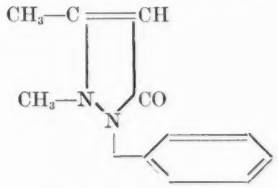
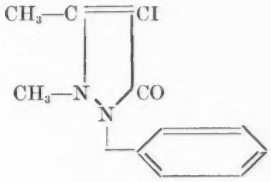
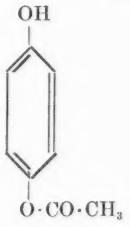
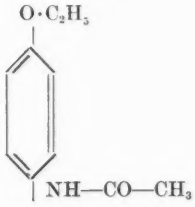
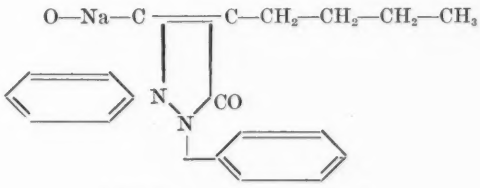
Miss Mon. was given injections, in the skin of the right and left forearms, of the progressively diluted A. B. serum. On the following day she ingested 0.5 Gm. of aminopyrine. The results are presented in Table III.

TABLE III

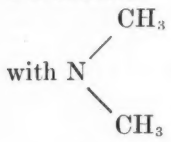
DILUTION OF SERUM	RESULTS
1:1	++++
1:4	++++
1:12	+++
1:24	++
1:48	+
1:96	±

7. *The Specificity of the Antigen-Antibody Reaction.*—The direct cutaneous tests applied with various chemically related substances have given the results shown in Table IV.

TABLE IV

SUBSTANCE TESTED	CHEMICAL STRUCTURE	REACTION
Pyramidon		+++
Antipyrine		+
Iodoantipyrine		++
Aspirin		–
Phenacetin		–
Butazolidin		–
Optalidon	Allyl-barbital : 0.05 Pyramidon : 0.125 Caffeine : 0.025	+++

Iodoantipyrine and aminopyrine are similar in that the carbon in position 3 is blocked



with N in aminopyrine and with I in iodoantipyrine.

Iodoantipyrine and antipyrine, when previously administered, were able in passive transfers to block the effect of aminopyrine. Aspirin and phenacetin were inactive. This shows that cross reactions occur only with pyrazolone derivatives.

8. *Attempts at Saturation of the Antibody by Addition of Aminopyrine in Vitro.*—We mixed 1 ml. of Patient A. B.'s serum with 0.5 ml. of a 1:100 solution of aminopyrine and then introduced it in a cellophane tubing. Dialysis was then performed against saline (ClNa 0.9:100). Dialysis was changed twice daily and continued for seventy-two hours to eliminate the possible excess of aminopyrine.

Intradermal injections of 0.1 ml. of the dialyzed serum and of 0.1 ml. of original serum (Patient A. B.) nondialyzed were made in the forearm of one of our subjects. Twenty-four hours later the subject ingested 0.5 Gm. of aminopyrine. After twenty minutes both sites were equally red and swollen. Both reactions were qualified ++++.

Fig. 3.—Thermolability of the drug antibody. On the left is shown the intradermal injection of unheated patient's serum (upper part) and of the patient's serum heated at 58° C. for thirty minutes (lower part).

On the right is shown the intradermal injection of patient's heated serum (upper part) and of a control serum (lower part). Twenty-four hours later, aminopyrine has been given orally. The reaction was positive (+++) with the unheated serum and negative with the heated and control sera.

9. *Physical and Chemical Properties of the Antibody.*—

(a) *Action of temperature:* The specific activity of the immune serum was perfectly well conserved by lyophilization at low temperature for more than five months.

In contrast, the antibody seems to be very sensitive to heat. The serum of Patient A. B. was heated to 56 degrees, 58 degrees, and 60 degrees for thirty minutes. Heating at 56 degrees destroyed about one-half of the sensitizing activity of the serum. Heating at 58 degrees and at higher temperatures completely destroyed the sensitizing properties of the serum (Fig. 3). The thermolabile properties of the antibody are similar to those of reagins.

(b) *The electrophoretic patterns of the antibody:* Recent studies by Sherman and Seebohm,[18] Cooke and associates,[3] Cann and Loveless,[2] and Seton and

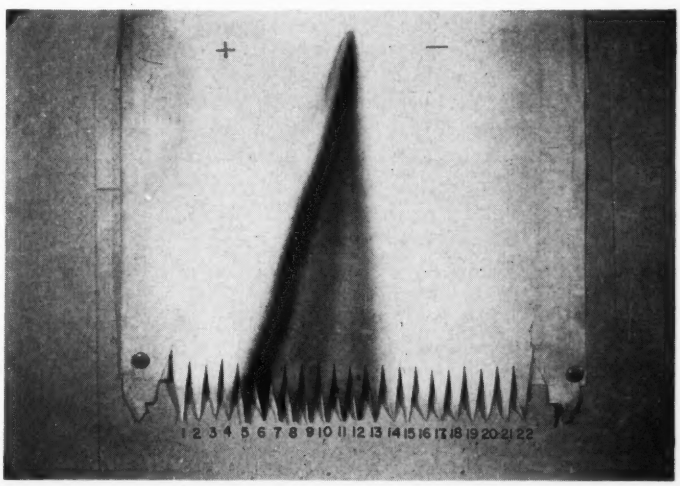


Fig. 4.—Electrophoretic separation of the serum fractions with the apparatus used by us.

Fig. 5.—Electrophoretic localization of the drug antibody.
On the left: Fraction 6 (serum albumin) negative
Fraction 8 (globulin γ) negative
Full serum of the patient ++++
On the right: Fraction 9 (globulin β_1) negative
Fraction 10 (globulin $\beta_2 + \gamma_1$) +++
Fraction 11 (globulin $\gamma_1 + \gamma_2$) ++

The fractions have been injected intradermally and the drug has been administered by mouth twenty-four hours later.

colleagues[17] have shown that the ragweed reagins are found in the β_2- γ_1 electrophoretic fractions of the human sera.

It was interesting to investigate the way in which this drug allergy antibody behaves in this respect.

We used the technique of continuous paper electrophoretic separation as described by Grassman and Hannig[8a] (Elphor V) (Fig. 4). Current applied was 140 volts, 20 milliamperes.

After forty-eight hours of electrophoresis, the eleven different fractions were collected, dialyzed, and tested on several nonallergic persons. Injections of 0.1 ml. of the various fractions were made intradermally, and aminopyrine was administered orally twenty-four hours later. The results are shown in Table V (Fig. 5).

TABLE V. ELECTROPHORETIC LOCALIZATION OF THE ANTIBODY

FRACTION NO.	NATURE	RESULTS
5, 6, 7	albumin	negative
8	globulin γ	negative
9	globulin β_1	negative
10	$\beta_2 + \gamma_1$	++++
11	$\gamma_1 + \gamma_2$	++

The specific antibody migrates, therefore, with the globulin fraction $\beta_2 - \gamma_1$ in a way similar to the pollen reagins.

DISCUSSION

The antibodies of our patient sensitized to aminopyrine have all the properties of the spontaneous reagins. The essential problem is whether aminopyrine acts as a full and complete atopen or as a hapten after conjugation with a protein. It is very likely that aminopyrine acts as a complete antigen. The immediate and urticarial type of reaction in the scratch test can hardly be explained otherwise. Aminopyrine is chemically weakly reactive and it is very unlikely, owing to its chemical constitution, that this compound may act only after conjugation with the small quantity of protein contained in the serosity of the scratch.

It is also unlikely that conjugation occurs when aminopyrine is given intramuscularly in passive transfer, even if such a hypothesis can be considered in the case of gastric administration.

How do we explain the failure regularly observed to produce a positive reaction when both antibody and antigen are injected intradermally? The most plausible explanation is that, due to the great diffusibility of aminopyrine, the substance does not remain locally in concentration high enough and also long enough to combine with the deposited antibody. The antibody-drug complex is likely to be very labile, as simple dialysis is sufficient to detach the chemical substance from the combination with the antibody. This has been well observed by Landsteiner,[10] who emphasized the considerable dissociation of the antibody-hapten complex. It is regrettable that no data are available in the literature on the liaison forces between the antibody globulin and chemical agents.

Erlenmayer and Berger[5] have studied experimentally the pattern of precipitation of rabbit sera injected with various pyrazolone derivatives. They

have studied the inhibitory action of various related substances on the precipitin formation and noted that this property is limited to the pyrazolone derivatives and that other agents, such as Veronal, salicylates, etc., are unable to interfere with the precipitation system. This is in agreement with our findings.

Another interesting point that should be emphasized is the rapidity with which the passively transferred antibody is fixed by the human skin; the skin reaction is maximal about four hours after the injection.

The clinical picture produced by the antibody-drug complex in our case is of a histaminic nature. The wheal-and-flare reaction is of urticarial form. The symptoms—prickling, urticarial blotches, and vascular collapse—should be attributed to a release of histamine. Moreover, the antihistaminics are able to control the symptoms produced in our patient by aminopyrine. It is worth while to emphasize this liberation of histamine by a combination of the antibody with a relatively simple chemical substance.

The immunologic features implicated in the case described by us are far from exceptional. O'Malley and Richey[13] reported a case of sensitization to quinine with positive skin tests. Dawson and Garbade[4] made a very interesting study on allergy to quinine, one of the authors being sensitized to this alkaloid. The skin tests have shown sensitization to all levogyre but not to the dextrogyre alkaloids of quinine.

Feinberg and Watrous[7] described a collective sensitization to chloramine T and Halazone in a factory with positive skin tests, and they conclude that chemical substances may react as complete allergens.

If the existence of processes of sensitization in which a chemical substance behaves as a full atopen is beyond any doubt, this mechanism is certainly not the most common. The most puzzling problem is aspirin allergy. Clinically, the fact is indisputable that aspirin allergy is humoral and its intensity is sometimes so severe that fatality can ensue. Nevertheless, the various attempts made to demonstrate the specific antibody, with a few exceptions,[9] failed in almost all cases.[8, 12, 16]

Perhaps aspirin allergy is one of the instances in which the antigen is a hapten which reacts only after conjugation with protein.

In fact, Butler, Harington, and Yuill[1] were able to produce precipitins in rabbits by sensitizing animals with aspirin conjugated with protein. This has been recently confirmed by Feinberg and Malkiel.[6] These authors sensitized both rabbits and guinea pigs by the injection of acetylsalicylic acid protein conjugates. The sera of sensitized rabbits showed precipitating antibody to aspirin as well as to sodium salicylate. Guinea pigs passively sensitized with serum from patients sensitive to acetylsalicylic acid could be shocked when challenged with heterologous acetylsalicylic acid plus protein conjugate.

The reason for the unsuccessful skin tests and passive transfers in many allergic conditions of the "anaphylactic type" may be double—insufficient amount of antibody and/or inadequate form of the antigen. It would be very desirable for new investigations to be made in this direction to clarify one of the most urgent problems. It was the aim of our study to help stimulate such investigations.

CONCLUSIONS

The investigations carried out on the immunologic features of the antibody in a patient sensitized to pyrazolone derivatives have led to the following conclusions:

1. The oral absorption of aminopyrine produces an anaphylactic type shock with vascular collapse.

2. The electroencephalogram made during this experiment has shown that the electrical alterations correspond to sudden vascular collapse (slow delta waves).

3. Cutaneous reactions, by scratch test, were strongly positive to aminopyrine and iodoantipyrine, slightly positive to antipyrine, and negative to Butazolidin, aspirin, phenacetin, etc. The reaction was of the immediate urticarial type.

4. Circulating antibodies could be demonstrated by passive transfer of the patient's serum into human beings.

5. The attempts at passive transfer into guinea pigs were unsuccessful.

6. In passive transfer the reaction can be elicited by either oral or parenteral administration of aminopyrine, but not by local injection at the site of the injection of the serum.

7. The antigen-antibody complex formed in vitro is labile and can be easily dissociated by simple dialysis.

8. The antibody is rapidly fixed in passive transfer and the reaction reaches almost the maximum as soon as four hours after the intradermal injection of the serum.

9. The antibody is thermolabile and is destroyed by heating for thirty minutes at 58° C.

10. The antibody migrates electrophoretically with $\beta_2 - \gamma_1 - \gamma_2$.

11. It is likely that the antigen-antibody complex is formed with the chemical substance, without any previous conjugation.

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ANTIGENIC RELATIONSHIPS BETWEEN HONEYBEES, WASPS, YELLOW HORNETS, BLACK HORNETS, AND YELLOW JACKETS

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A STING by some member of the hymenoptera group of insects produces in all victims local pain, redness, and some swelling that passes harmlessly in a few hours. After repeated stings, some persons develop altered reactions, ranging from exaggerated local swellings to severe systemic symptoms or sudden death. These systemic symptoms are not due to toxic effects of the venom, but are manifestations of allergic sensitization to substances in the venom. Patients sensitive to the sting of certain insects, in addition often have allergic inhalant symptoms when exposed to dust from bodies of the same insects. The sensitizing substance is probably protein in nature and found in venom as well as other parts of the insect's body. Desensitization with venom or extracts of whole insect bodies has proved effective in controlling these severe allergic reactions.[1-4]

Desensitization entails administering, in graded dosages, the antigen to which the patient is allergic. The high degree of specificity of the antigen-antibody reaction necessitates accurate identification of the sensitizing allergen. If the offending insect is known, the antigen to be used for desensitization is obvious. Often, however, stings and reactions take place without identification of the assailant. Several questions then arise: Extracts of what insect or group of insects will effectively desensitize a patient to all possible stings? Must each specific insect be used for desensitization, or will one insect effectively desensitize to all others?

Skin testing with insect venom or extracts, while sometimes a helpful procedure, does not give detailed information about the number and relationship of antigens present. The present investigation was undertaken to seek some answers to these questions.

MATERIALS AND METHODS

Only a relatively few members of the hymenoptera order are important in causing stings. For accuracy in collection and identification, this study was limited to those insect species of clinical significance in eastern Washington and northern Idaho.

The species used in this work were:

Wasps: *Polistes fuscatus aurifer*
Hornets: *Dolichovespula arenaria*—yellow
Dolichovespula maculata—black

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Yellow jacket: *Vespula pennsylvanica*
Honeybee: *Apis mellifica*—(German honeybee)

These same insects, or closely related species, are responsible for the great majority of stings throughout North America.

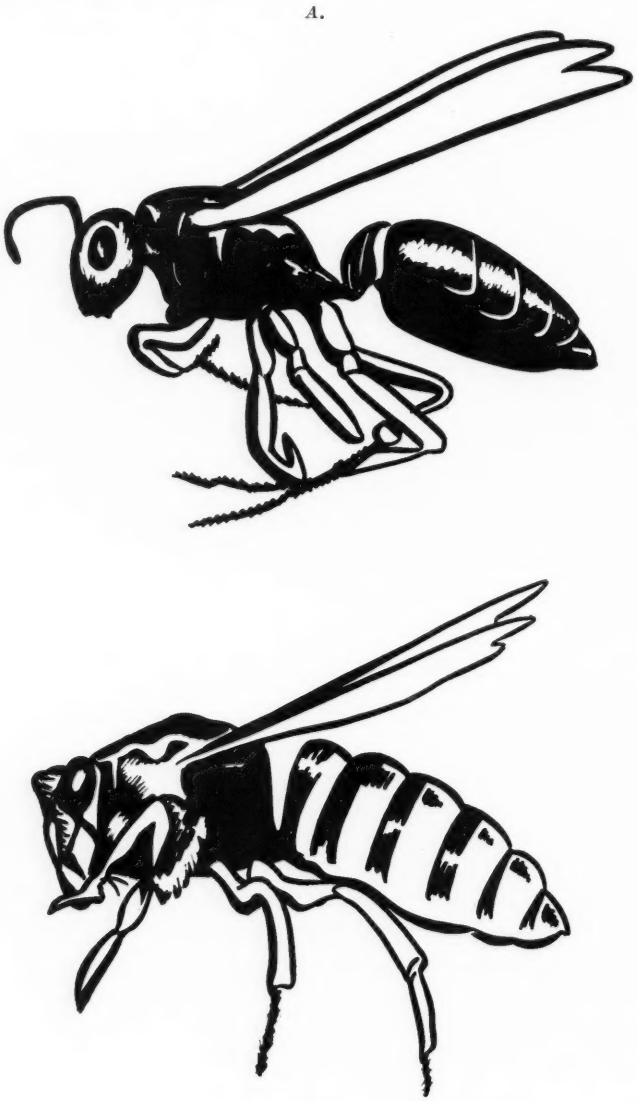


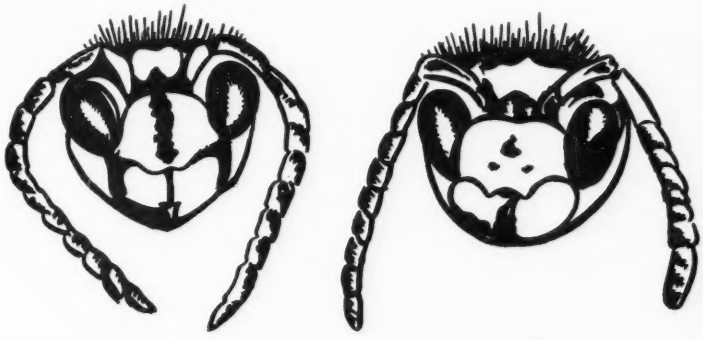
Fig. 1.—*A*, Wasp with fusiform abdomen and elongated, narrow waist. *B*, Hornet and yellow jacket with truncated abdomen and short waist.

A short review of the identifying characteristics of these insects is in order.

The wasps (*Polistes*) are easily identified by the fusiform abdomen (Fig. 1), gently tapered at both ends and joined to the thorax by a definite narrow waist.

They are hairless. In color, they are black or marked with other colors, usually red, yellow, or white. Wasps marked with yellow are called yellow jackets in certain localities. Their nests are flat, open combs in sheltered places, such as eaves, porches, barns, and garages.

The hornets (*Dolichovespula*) and yellow jackets (*Vespula*) are closely related and are identified by the abdomen, which is sharply truncated anteriorly (Fig. 1) and attached to the thorax by a very short waist. They are more hairy than wasps and are black marked with white or yellow. The presence of yellow markings does not distinguish the yellow jacket, since there are also yellow hornets. The distinguishing feature of the hornet is a definite extra black plate between the lower margin of the eye and the mandible (Fig. 2), giving a long-faced appearance. The yellow jacket lacks this plate and the mandible articulates at the lower margin of the eye. The most convenient distinguishing feature is the difference in habitat. The hornet makes a typical football-shaped nest well above the ground, while the yellow jacket builds its nest under the ground, in rocks, or between walls of houses or barns.



A. *B.*

Fig. 2.—*A*, Hornet with black bar between eye and mandible. *B*, Yellow jacket with eye and mandible directly articulating.

The bees, with their stocky hairy bodies and round abdomens, are familiar. However, in some areas, wild black bees may be confused with hornets.

Bumblebees were not included in this study, since they nest in small underground colonies and were impossible to collect in sufficient numbers to make an extract.

Yellow jackets were collected either in traps or with the hose attachment of a tank type vacuum cleaner. Other insects were collected at night in their nests. The nest was quickly enclosed in a plastic container and then detached from its support. This material was transferred to plastic bags and placed in a freezer at –20° C. Undamaged insects were picked out of the debris and stored in the freezer until used.

Antigens were prepared by extracting 5 grams of pulped insect bodies in 100 ml. of saline containing 1:10,000 Merthiolate. The Freund adjuvants-antigen

mixture was made from 50 mg. each of *Mycobacterium phlei* and *Mycobacterium smegmatis,* 20 ml. light paraffin oil, 10 ml. Aquaphor, and 10 ml. of saline antigen extract.

Alum-precipitated antigen was prepared by adding 10 ml. of 5 per cent ammonium aluminum sulfate to 10 ml. of saline insect extract. The pH was adjusted to 6.5 with N. sodium hydroxide. The resultant precipitate was separated by centrifugation and resuspended in 10 ml. of saline and Merthiolate.

A prolonged course of sensitization was employed in order to develop antibodies in as high titer as possible to as many antigens as possible.[5] Rabbits were sensitized at first, with 1 c.c. of the adjuvant-antigen mixture injected subcutaneously each week. After three or four injections, marked Arthus reactions occurred. Thereafter, 1 c.c. of alum-precipitated extract was injected intraperitoneally every two weeks. After the second injection, bleedings of 10 to 50 c.c. of heart blood were begun. Bleedings were continued every two weeks, either ten days after injection of the Freund adjuvant-antigen mixture or three days after injection of the alum-precipitated antigen. A precipitin titer was determined on the serum from each bleeding, and only those sera of high titer were stored at –20° C. for use in the following experiments.

Gel diffusion studies were carried out in Petri dishes according to the method outlined by Ouchterlony.[6] The homologous and one heterologous antigen were diffused against rabbit antiserum on each plate. Plates were kept in the refrigerator at 4° C. until the precipitate lines were fully developed. The plates were examined every two weeks and drawings were made of the lines of precipitation. When the lines had completely developed and were no longer changing, the plates were photographed.

Guinea pigs were sensitized by injecting 0.5 c.c. of alum-precipitated antigen intramuscularly each week for three weeks. Twenty-one days after the last sensitizing injection, the guinea pigs were challenged with 2 c.c. of saline extract injected intracardially. One unsensitized control animal was injected for each five sensitized animals. None of the extracts produced any symptoms whatever in control animals. All animals that died after a challenging injection of antigen were autopsied. Of 140 dead animals examined, seven showed hemorrhage from the cardiac puncture or other findings not typical of the distended, air-containing lungs of anaphylaxis and were discarded from the series.

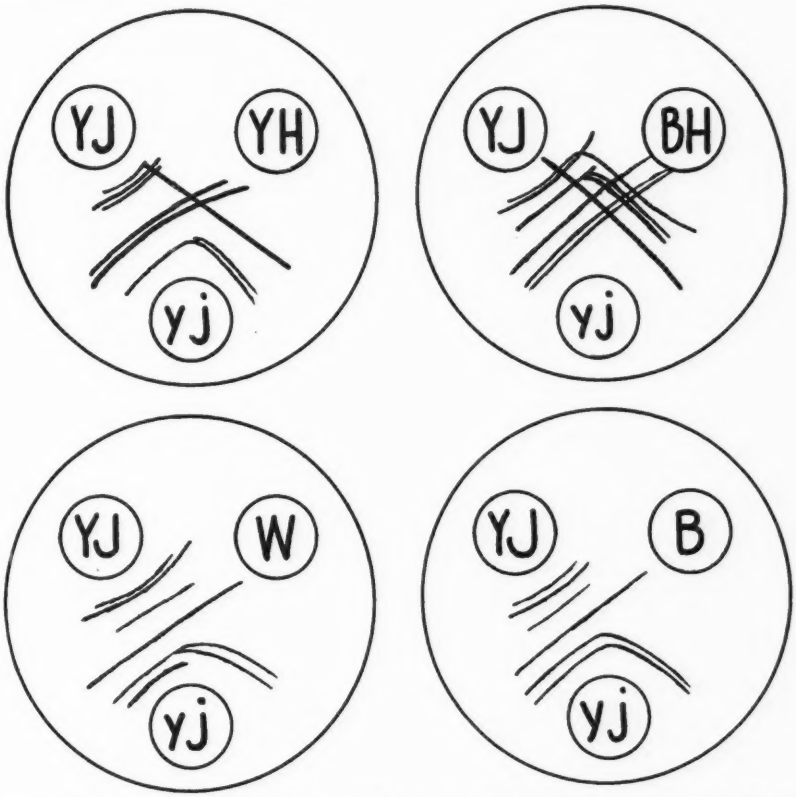
RESULTS

Gel Diffusion Studies.—The drawings made from Petri dishes at the maximum development of precipitate lines are shown in Figs. 3 to 7.

In Fig. 3 yellow jacket antiserum (*yj*) was diffused against extracts of yellow jacket (*YJ*), yellow hornet (*YH*), black hornet (*BH*), wasp (*W*), and honeybee (*B*). Clearly, yellow hornet and black hornet extracts showed at least three antigens shared with yellow jacket extract, while bee and wasp extracts showed only one or two.

In Fig. 4 anti-yellow hornet serum (*yh*) was reacted with the same five insect extracts. All extracts demonstrated at least one antigen in common; yellow jacket, black hornet, and yellow hornet demonstrated at least three, as in the previous experiment.

In Fig. 5 anti-black hornet rabbit serum (*bh*) was diffused against the five insect antigens. Once again an antigen was found to be held in common by each insect, although the common antigen of the bee extract must be present in very small concentrations. Only the yellow hornet extract produces evidence of more than this one antigen in common. However, black hornet antigen was shown to include several additional antigens contained in extracts of yellow jacket and yellow hornet (Figs. 3 and 4). Possibly the black hornet extract is not as capable of stimulating antibody production as are yellow jacket or yellow hornet extracts.



Figs. 3 to 7.—Ouchterlony type gel diffusion plates. Antigens in upper wells (capital letters); antisera in lower well (lower-case letters). *YJ* and *yj*, yellow jacket; *YH* and *yh*, yellow hornet; *BH* and *bh*, black hornet; *W* and *w*, wasp; *B* and *b*, honeybee.

In Fig. 6 the five insect extracts were diffused against antiwasp serum (*w*). At least one antigen occurred in each extract that reacted with wasp antiserum, although yellow jacket extract gave evidence of at least three. Wasp extract contained a number of additional reactants not present in the other four insect extracts.

In Fig. 7 the antibody used was the serum of rabbits sensitized to bee extract. One or two antigens from each insect extract were found to react with bee antiserum (*b*). None of the remaining antigens in bee extract were found in extracts of yellow jacket, black hornet, yellow hornet or wasp.

Figs. 3 to 7 suggest that probably the same one or two rapidly diffusing antigens are found in each insect extract. Yellow hornet, black hornet, and yellow jacket extracts gave the best evidence of additional antigens in common. Each genus of insect tested contained a number of antigens that were specific for itself and not shared with other insects. Of all the insects investigated, the

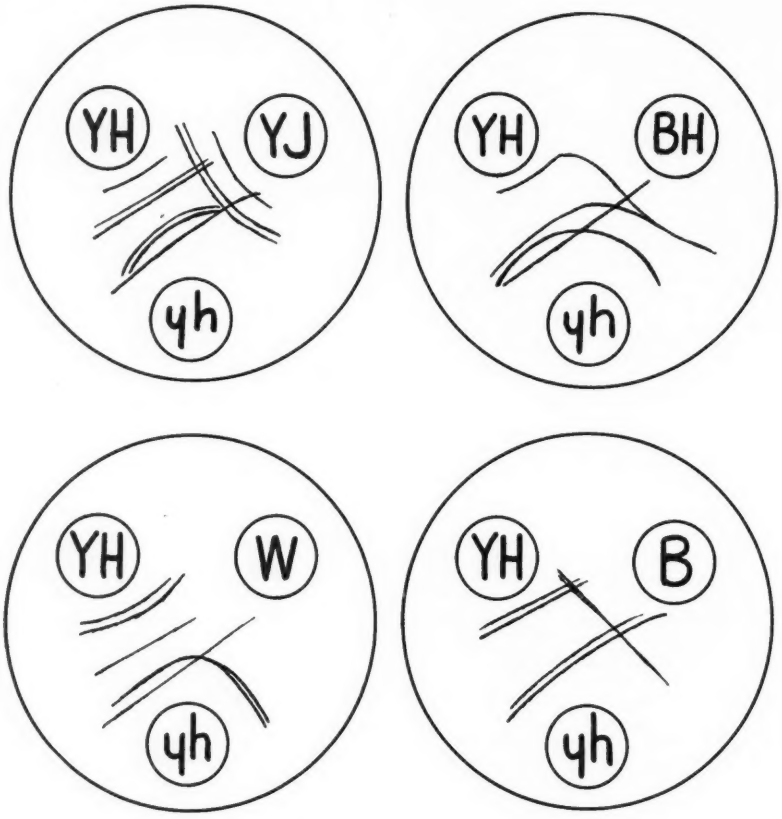


Fig. 4.—*(For explanation, see Fig. 3.)*

yellow jacket produced the antibody that most readily reacted with heterologous antigens. Yellow jacket extract also showed the greatest tendency to cross react with heterologous antisera. It should be noted that there is very little correlation between the titers of antigen or antibody and the amount of cross reaction displayed among these antigens when this technique is used.

Anaphylactic Shock Studies.—Fig. 8 presents the results of the anaphylactic shock experiments in guinea pigs. In almost every experiment, the homologous antigen precipitated fatal shock in every guinea pig. The exception

was black hornet antigen, which caused fatal shock in four of six black-hornet-sensitized animals. Except for the yellow-jacket-sensitized animals, heterologous antigens produced a greater incidence of survival from anaphylaxis. The bee-sensitized, yellow-hornet-challenged animals uniformly showed no symptoms of shock whatever. However, when yellow-hornet-sensitized animals were injected with bee antigen, some degree of shock invariably occurred. This suggests that yellow hornet extract produces better sensitization than does bee antigen. Since heterologous antigens invariably produced fatal shock when injected into yellow-jacket-sensitized animals, yellow jacket antigen would seem to have the greatest ability to produce sensitization to other stinging insects. Bee antigen, however, would appear to have the least ability to produce sensitivity to the heterologous

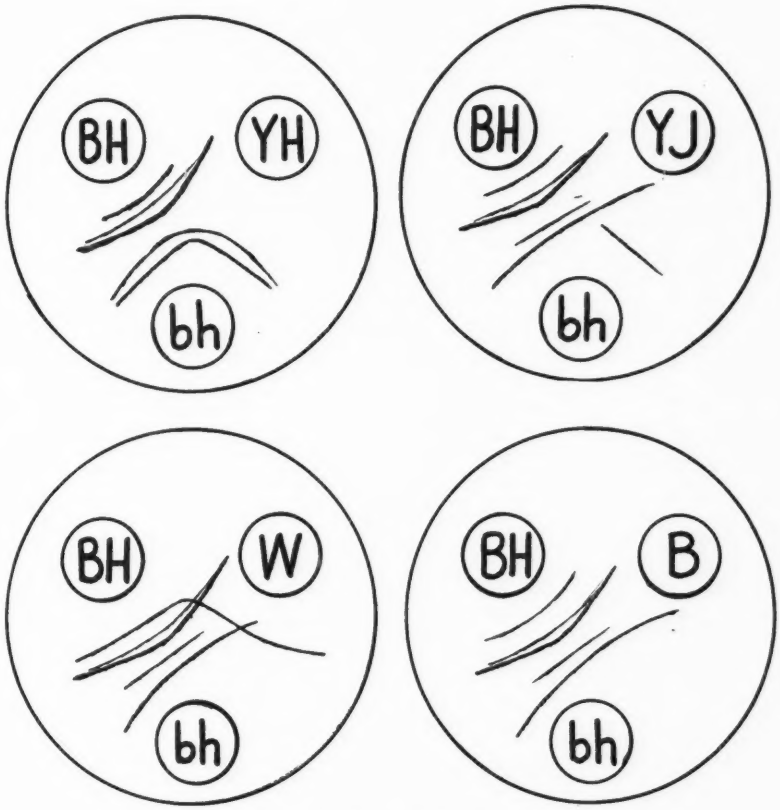


Fig. 5.—*(For explanation, see Fig. 3.)*

antigens. The closest relationship to bee antigen would seem to be wasp. Black hornet appears to have less ability to produce sensitivity to heterologous as well as homologous antigens. Except for yellow jacket, all the insect antigens produced a less consistent sensitization to heterologous antigens.

In general, the hornet group (yellow jacket, yellow hornet, and black hornet) produced a high incidence of clinical shock as well as fatal shock in animals sensitized to heterologous antigens of this same group.

CONCLUSIONS

Gel diffusion studies indicate that bee, wasp, yellow hornet, black hornet, and yellow jacket extracts contain from four to six antigenic fractions. Probably two of these are immunologically identical in all of these insects. However, the remaining two to four antigenic fractions are specifically characteristic of the individual insect species and are not shared with other species. Benson[7]

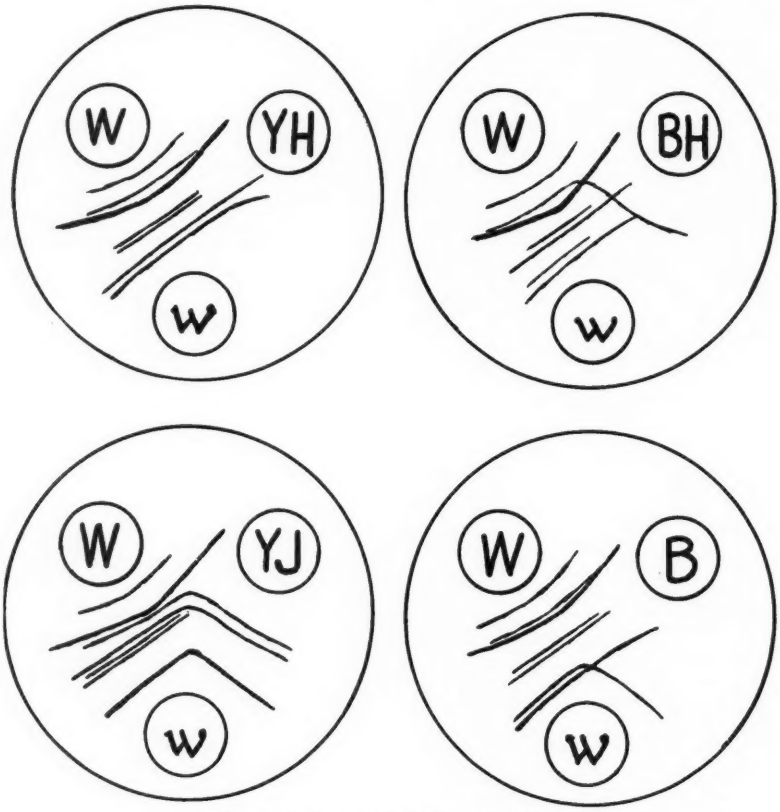


Fig. 6.—*(For explanation, see Fig. 3.)*

documented patients who had reactions to stings of bees but no reaction to wasp or hornet stings. Similarly, some patients displayed skin reactions to one stinging insect and not to others. This indicates that man can become sensitized to individual antigenic fractions to the complete or partial exclusion of others. Sensitization may be to antigens shared by all stinging insects, to antigens contained in only a single species, or to antigens of both groups. To conclude that

extracts of a single insect would effectively desensitize all patients to all other stinging insects would, therefore, be wrong. If the clinical experience of a sensitive patient indicates (1) that the shocking insect was accurately identified and (2) that the patient suffered no reaction when stung by other insects, then the use of a single insect antigen should be safe. In most cases, neither provision can be fulfilled. Therefore, to assure adequate protection for the patient, an antigen should be used which contains at least the extracts of honeybee, wasp, yellow jacket, and hornet. It is, of course, important that the insects be accurately identified before extraction.

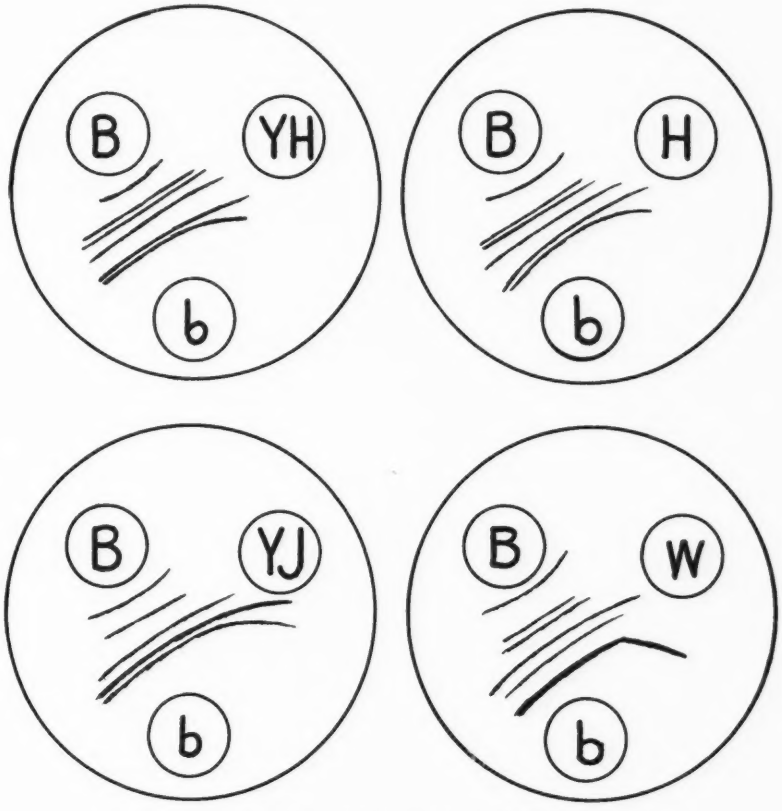


Fig. 7.—*(For explanation, see Fig. 3.)*

Anaphylactic shock experiments with guinea pigs suggest that yellow jacket extracts are the most potent in producing sensitization to shock from heterologous antigens. Yellow jacket extract also was most effective in shocking animals sensitized to the heterologous insect extracts. Bee extracts, however, were considerably less able to produce sensitization or shock to heterologous antigens. If there is any relationship between the mechanism of anaphylactic sensitization in guinea pigs and of spontaneous allergic or anaphylactic sensitization in man, then yellow jacket extract would be the most important insect antigen to include in a desensitization mixture.

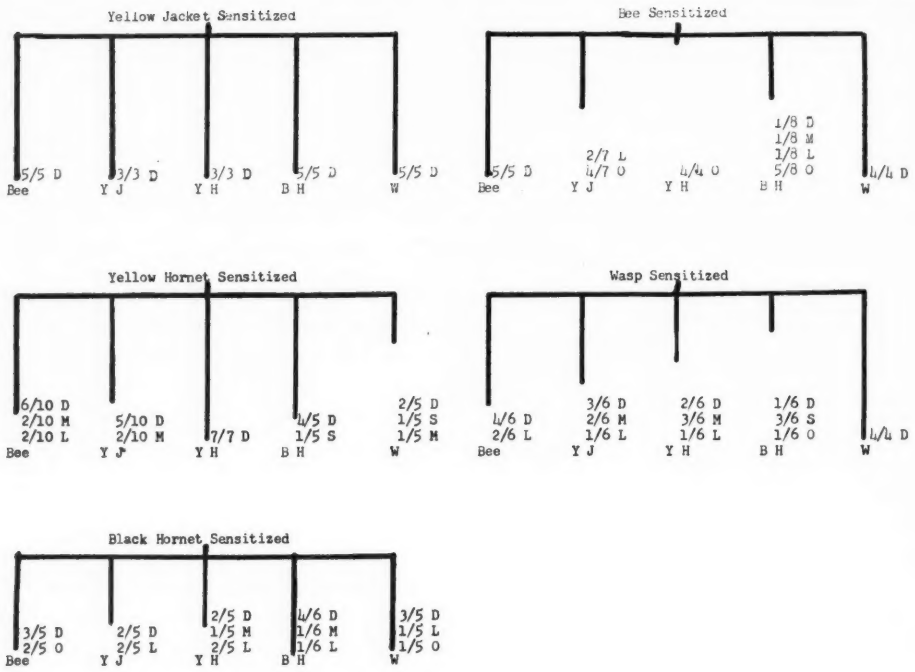


Fig. 8.—Anaphylactic shock in guinea pigs. *YJ*, Yellow jacket; *YH*, yellow hornet; *BH*, black hornet; *W*, wasp; *D*, died of anaphylactic shock; *S*, severe shock with survival; *M*, moderate shock with survival; *L*, light shock with survival; *O*, no reaction. Unreported are those which died from causes other than anaphylactic shock.

SUMMARY

1. Gel diffusion studies indicate that yellow jacket, yellow hornet, black hornet, wasp, and honeybee contain common antigens. In addition, each insect contained several antigens specific for the individual genus.

2. Anaphylactic shock experiments corroborate the relationships suggested by the gel diffusion studies. Homologous antigen produced fatal anaphylactic shock in all guinea pigs challenged except those sensitized to black hornet. Heterologous antigens produced lesser degrees of shock or no shock whatsoever. The antigens varied in their ability to produce sensitization. Yellow jacket antigen was the most potent sensitizer, while black hornet antigen was the least potent sensitizer. Animals sensitized to bee antigen demonstrated the least degree of heterologous sensitization.

3. If a patient has a severe allergic reaction to an insect sting and the offending insect can be identified accurately, then desensitization with the single insect may be safe. However, if the insect causing a severe reaction cannot be identified, or if there is any possibility of sensitivity to other stinging insects, then desensitization with a combined antigen would seem to be advisable.

These data suggest that a satisfactory antigen mixture should include at least bee, wasp, yellow jacket, and hornet extracts.

The authors wish to express their appreciation to Karl V. Krombein, Entomology Research Branch, U. S. Department of Agriculture, Washington, D. C., for his cooperation in identifying the insects used in these studies.

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THE VALUE AND LIMITATIONS OF MOLD SURVEYS BY THE CULTURE PLATE METHOD

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THE present plate technique[1] in making mold surveys consists of exposing at about the same time each day a standard Petri dish covered with modified Mehrlich's medium. According to the 1949 report of the Subcommittee on Mold Survey of the Research Council, American Academy of Allergy,[2] "The only conclusion to be drawn at this time is that slide counts and plate counts for *Alternaria* are, by and large, in rather poor agreement. This is known to be true for *Hormodendrum* as well."

As the plate method has been arbitrarily chosen as a standard,[2] it was hoped that further study of this method might be of value in the interpretation of results reported in terms of this standard plate method.

PROCEDURE

A series of readings was taken daily, beginning at 11 o'clock each morning. A Petri dish containing modified Mehrlich's medium was exposed on the top plane of a standard gravity sampler.[3] During the exposure of this Petri dish (twelve minutes), an air sample was taken by a sampler utilizing both electrostatic precipitation and impingement in the collection of air-borne spores.[4] Immediately following this twelve-minute exposure, a second Petri dish was exposed at the same sampling site and for the same length of time. During the exposure of this second Petri dish, a second air sample was taken by the same air sampler, utilizing only simple impingement as the sampling method.

The intake into the air sampler was at the same level as the exposed Petri dish and about 1 foot distant from the exposed plate. The rate of air flow in all readings was 0.5 cubic foot per minute. As the instrument was in continuous operation during the exposure time of the Petri dish, each air sample represented 6 cubic feet of air. A flat roof was selected for the sampling site with no obstructions to the passage of lateral air currents over the exposed plate.

Eighty-eight daily readings were taken during the months of April, May, June, and July. The four culture plates representing a daily reading, two by the standard plate method and two by a volumetric sampler, were immediately placed in an incubator. The plates were incubated for seven days at a temperature of 85° F. The plates were then removed and the colonies were identified and counted. This study was limited to hormodendrum and alternaria because of their high incidence and their importance in producing allergic manifestations.

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DISCUSSION AND RESULTS

The Sampling Efficiency of Electrostatic Precipitation Plus Impingement as Compared With Simple Impingement.—Figs. 1 and 2 indicate a comparatively low sampling efficiency of the air sampler used by simple impingement. These results are similar to those of a previous report in a study of air-borne penicillium spores. In that study[4] it was found that the addition of electrostatic

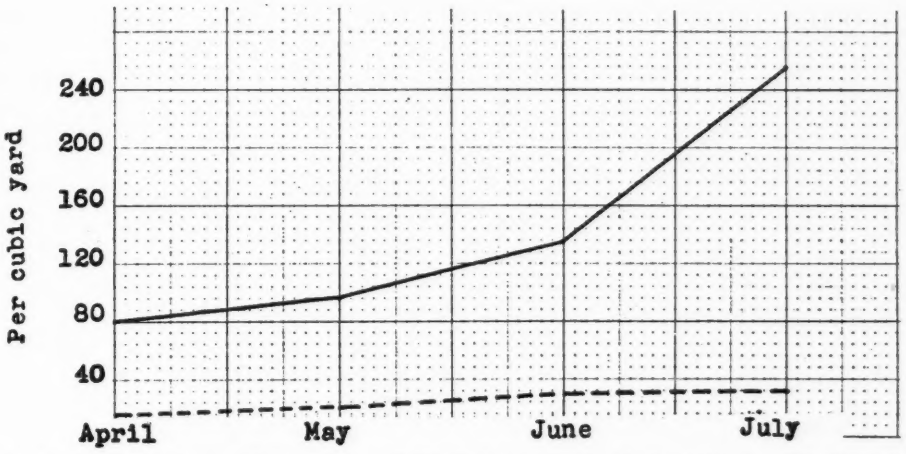

Fig. 1.—Hormodendrum colony count. Solid line represents electrostatic precipitation plus impingement; broken line represents impingement.

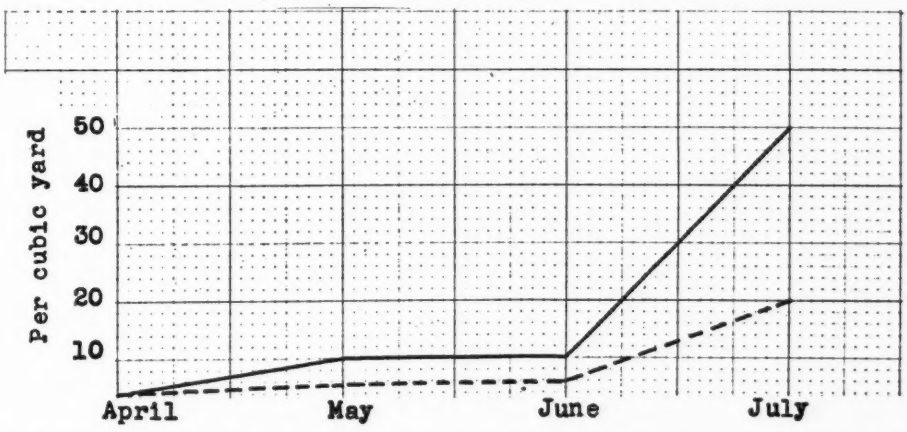

Fig. 2.—Aternaria colony count. Solid line represents electrostatic precipitation plus impingement; Broken line represents impingement.

precipitation to impingement markedly increased the sampling efficiency of the same instrument and, under the experimental conditions cited in the study, it was believed the sampler utilizing electrostatic precipitation approached a 100 per cent sampling efficiency.

In this comparison of efficiency (Figs. 1 and 2), the sampling error for hormodendrum is greater by impingement than for alternaria. This is to be

expected because of their smaller size and, similarly, in sampling by the culture plate method the sampling error for hormodendrum must be greater than for alternaria.

A Daily Comparison of the Number of Hormodendrum and Alternaria Colonies Cultured on Two Exposed Petri Dishes Exposed at Different Time Intervals.—The number of spores collected by an exposed plate for twelve minutes, as compared to the number of spores that are caught during a second twelve-minute exposure of another culture plate, would depend primarily on two factors: (1) the extent to which there is a homogenous mixture of the spores in a given volume of air and (2) the velocity of air flow over the exposed plate or air turbulence.

If there is a homogenous mixture of these spores in the air, the ratio of the number of spores collected by electrostatic precipitation plus impingement to the number collected by simple impingement would remain about the same. However, daily comparisons of these volumetric readings indicated a marked variation in the number collected by the two volumetric methods. On a few days, the number of alternaria spores collected by electrostatic precipitation plus impingement was about the same as that sampled by simple impingement, while on other days eight times more were collected by the first method as compared with the second method. Similarly, in the sampling of hormodendrum spores, although on all days the number sampled by electrostatic precipitation and impingement was greater than the number collected by simple impingement, the ratio changed from three times as many on one day to a high of twenty times as many on another. These air-borne spores are, as in the case of air-borne ragweed pollen, coming in waves.

Although there was a lapse in time of only 12 minutes between exposure of the first and second culture plates, sampling conditions would not necessarily be the same.

One would hardly expect much agreement, then, in the daily totals of spores collected by two culture plates exposed at the same sampling site but at different intervals of time. On some days there was close agreement in the total count, while on other days seven times as many alternaria spores were collected by one plate as compared with the other. On other days nine times as many hormodendrum spores were collected by the one plate as compared with the other. The spore count reported in terms of the present standard plate technique does not reflect with any degree of accuracy the actual incidence of alternaria and hormodendrum spores for a given day, and a sudden daily increase or decrease in the number of spores collected by this method is of no significance.

A spore count made with a volumetric sampler would have little more meaning than the present culture plate method if short time runs were used. This is because of a lack of uniformity in the number of spores present in a given volume of air.

A Monthly Comparison of the Number of Hormodendrum and Alternaria Spores Collected by Two Exposed Culture Plates.—Figs. 3 and 4 indicate that

the total number of spores collected by two exposed plates was about the same when monthly totals were computed and when these totals were expressed in terms of a daily average. These readings suggest that the culture plate method does have value in that it indicates at least a seasonal trend in the incidence of these two spores. It would also appear likely that monthly totals expressed in terms of a daily average are of some value in a comparison of incidence of these spores in one area as compared with another.

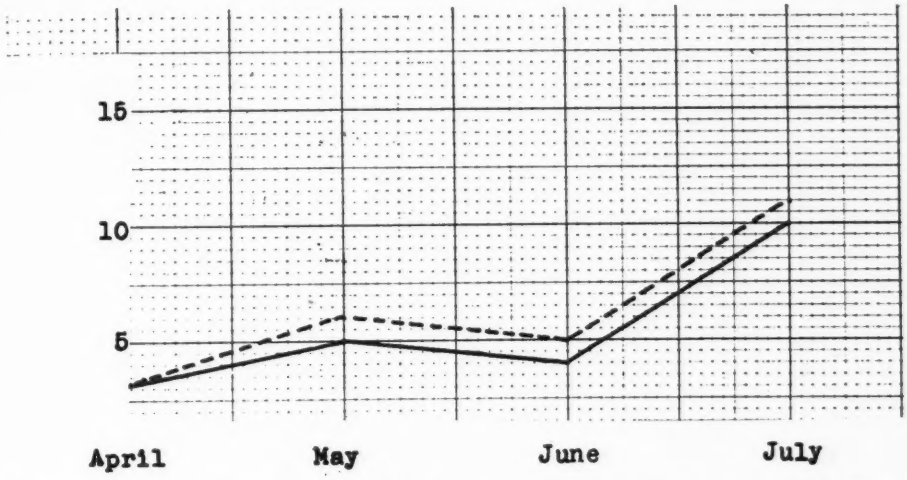

Fig. 3.—Alternaria, daily average. Solid line represents Plate 1; broken line represents Plate 2.

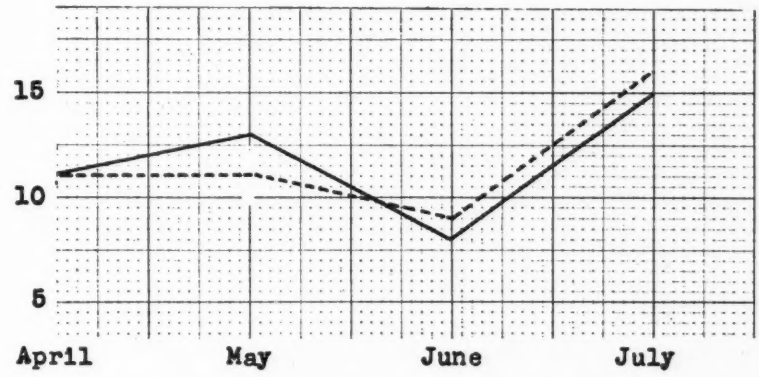

Fig. 4.—Hormodendrum, daily average. Solid line represents Plate 1, broken line represents Plate 2.

SUMMARY

A total of eighty-eight readings were taken during the months of April, May, June, and July in an attempt to evaluate the standard culture plate method in sampling for alternaria and hormodendrum spores.

Each daily reading consisted of the exposure of two culture plates at the same time of day but at slightly different time intervals. During the exposure

time of these plates, a known volume of air was sampled at this location by an instrument utilizing electrostatic precipitation with impingement. Additional air samples were taken by just impingement with the same instrument.

1. The application of electrostatic precipitation to simple impingement in the sampling instrument used increased the sampling efficiency for both alternaria and hormodendrum spores.

2. The daily colony count by the standard plate technique does not reflect the true incidence of alternaria and hormodendrum spores for a given day.

3. These readings suggest that when monthly totals are computed of the spores collected and these totals are expressed in terms of a daily average, the results do indicate a seasonal trend in the incidence of air-borne alternaria and hormodendrum spores.

4. Short-time volumetric air samples are of little value in determining the actual incidence of air-borne alternaria and hormodendrum spores, because of a lack in uniformity of spores present in a given volume of air.

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STUDIES WITH SPUTUM

I. Initial Observations on the Chemical Nature and Blood Group Substance Content of Asthmatic Sputum

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THE intrabronchial accumulation of a peculiarly thick, viscid mucus has long been recognized as a characteristic defect of the variety of non-extrinsic asthma variously designated as intrinsic or infective asthma.[1-8] The accumulation of such viscid mucus is not confined to the asthmatic patient but is frequently encountered in such other chronic diseases of the respiratory tract as chronic bronchitis, certain instances of emphysema, tuberculosis, and cystic fibrosis and is seen following a variety of neuromuscular disorders, such as poliomyelitis, muscular dystrophy, and amyotrophic lateral sclerosis. The degree to which such mucus may obstruct the airways is extremely well illustrated in gross specimens and in sections of the lungs of persons who have died in asthmatic attacks (Fig. 1). Interest in the chemical nature of this mucus developed for a number of reasons. First, it seemed of importance to ascertain whether there were significant chemical differences in the mucus associated with various disease states and whether any or all of these mucous secretions were significantly different chemically from the mucus normally present in the respiratory tract. Second, it seemed desirable to pursue such chemical studies as an initial approach to the broader physiologic problem of defining the mechanisms of intrabronchial mucous accumulation. An additional stimulus to the pursuit of these studies came from the publication of a number of articles dealing with the clinical use of various enzyme or detergent aerosols for the reduction of the viscosity of respiratory secretions.[9-32]

Almost coincidentally with the first steps of the present investigation, Werner[33] published an extremely extensive study on the glycoproteins from mucous epithelium and epithelial secretion. His section on the respiratory tract reviewed the meager data on the chemistry of respiratory secretions preceding his own work. In 1901 Müller[34] had applied a complex purification procedure to sputa collected from persons suffering from various kinds of bronchial and pulmonary disease. He succeeded in obtaining samples of "pure" mucin containing between 20 and 35 per cent reducing substances liberated by acid hydrolysis. From this he isolated glucosamine hydrochloride and osazone which was probably galactosazone. Werner's investigations confirmed Müller's findings and established, by paper chromatography, the presence of galactose. All of three specimens of sputum obtained by Werner

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from a person suffering from acute bronchitis contained hexosamine, galactose, fucose, and traces of mannose. Neither sulfate ester groups nor hexuronic acid were found in any of the specimens. Amino acid chromatography

Fig. 1.—Gross specimen of lung of patient who died in an acute asthmatic attack. The forceps are holding a stretched strand of the homogeneous, acellular, bronchial mucus which literally filled the bronchial tree. (Courtesy of Merle S. Scherr, M.D., Charleston, West Virginia.)

of one of the sputum specimens revealed no unusual features, with the possible exception of some increase in the amounts of threonine and phenylalanine. In fact, the latter finding induced Werner to hypothesize that these amino acids might be involved in conjugation with the polysaccharide moiety, although he did not speculate as to the nature of such bonds. Neither Werner nor Müller made any observations on the biologic activity of their preparations, despite the fact that Werner recognized that the predominant glycoprotein in epithelial mucus was composed of hexosamine, galactose, and fucose and was thus of the same type as that of the purified blood group substances.

The present studies included assay of blood group activity of all fractions prepared during purification, since it was thought necessary to exclude the possibility that blood group substance itself might be responsible for the viscosity of sputum. In this regard, a recent article by Springer, Rose, and Gyorgy[35] reported that bronchial mucus is capable of inhibiting A, B, and H agglutinins and is also active in promoting growth of *Lactobacillus bifidus var. Penn.*, whose growth factor has been shown to consist of nitrogen-containing oligosaccharides and polysaccharides which have as building stones the same simple sugars as blood group mucoids.

The present report is concerned with the initial purification procedures and analytical techniques employed in a pilot study of the combined alcohol precipitates from twenty-eight sputa. The data obtained on chemical analysis of some of the individual sputa before they were pooled will also be presented.

METHODS

A. *Initial Treatment and Chemical Analysis of Sputa.*—Specimens of sputum were collected at the bedside in standard sputum boxes or wide-mouthed glass jars. No particular washing procedure was applied to the specimens. In virtually all instances, the specimens were thick, extremely viscid, and contained nummular masses of opaque material. Smears were made of each sputum specimen and were stained by Gram's method. Thirty-one individual specimens form the subject of this report; the major portion of the dried 65 per cent alcohol precipitates of the first twenty-eight of these were combined in a 25 gram pooled precipitate with which the experiments of the present report were performed.

The individual sputa were diluted with an equal volume of distilled water and then precipitated with ethanol at a final concentration of 65 per cent. The precipitates were successively washed with 95 per cent ethanol, absolute ethanol, and acetone, and then were dried under vacuum. Each fraction was weighed, and samples of each were brought to a concentration of 2.0 mg. per milliliter and analyzed for carbohydrates,[36, 37] nitrogen,[38, 39] and hexosamine.[40]

Chromatographic analyses were carried out for amino acids with samples containing 0.25 mg. nitrogen[41] and for carbohydrates[42, 43] with samples containing 300 gamma total carbohydrate.

B. *Phenol Extraction and Pepsin Digestion.*—The alcohol-insoluble fraction of sputum No. 7 was extracted with 90 per cent phenol three times; the three phenol-soluble fractions were combined and then precipitated stepwise with ethanol at 10 to 65 per cent concentration. The precipitates obtained at the 10 per cent concentration of ethanol gave evidence of purification in that carbohydrate and hexosamine content increased while the nitrogen decreased. The yield, however, was small and the final product was not very viscous. The phenol-insoluble residue was approximately 15 per cent soluble in water and, after drying, this fraction remained completely soluble in water and was somewhat viscous.

In the effort to increase the yield of active material, the alcohol-insoluble precipitates of sputum were next exposed to pepsin after the method of Bendich, Kabat, and Bezer.[44] For this purpose, the precipitates obtained from twenty-eight sputa were combined to form a 25 gram pool of starting material which will hereafter be referred to as CI.

We added 1.0 mg. of twice recrystallized pepsin to 75.0 ml. of a sodium citrate-hydrochloric acid buffer at pH 2.3 (50 ml. of 0.1M sodium citrate + 150 ml. 0.1M hydrochloric acid). We then added 75.0 ml. of this pepsin buffer to a 10.0 Gm. aliquot of CI in a 500 ml. Erlenmeyer flask. A control flask contained 10.0 Gm. of CI and 75.0 ml. of the buffer at pH 2.3 without added

pepsin. Both flasks were kept in a warm room at 37° C. for nine days with adjustment of each back to pH 2.3 by addition of acid on the third day, at which time another 1.0 mg. of pepsin was added to the mixture. On the fifth day 1.0 mg. of pepsin was added to the whole pepsin mixture, and on the seventh day 1.0 mg. was added to the centrifuged sediment of the pepsin digest, which was then returned to its flask with 40 ml. of fresh buffer and incubated for another two days. The supernatant from this procedure was combined with the final supernatant.

At the end of this period, the supernatants were obtained from each incubated mixture by centrifugation in the Servall Superspeed SSI centrifuge at 16,000 r.p.m. for 20 minutes at 4° C. The supernatant from the pepsin digest was almost twice the volume of that from the buffer digest alone and was moderately viscous. Each supernatant was opalescent and was saved for the precipitation with alcohol.

The residues were washed twice with 40.0 ml. of buffer at pH 2.3 and the washings were combined with their respective original supernatants, while the residues themselves were washed and dried in alcohol and ether, placed under vacuum, and stored in the dry state for later study. A similar process was carried out with a 1.0 Gm. sample of CI in 7.5 ml. saline at pH 7.0. The partitioning of the three digests is given in Table I. Pepsin digestion increased to 92 per cent the final supernatant yield, as compared with 74 and 82 per cent in the control flasks.

TABLE I. PARTITION BY PEPSIN DIGESTION

	PEPSIN (GM.)	ACID (GM.)	SALINE (GM.)
Original weight	10.0	10.0	1.0
Supernatant			
Total recovery	9.9766	9.7596	0.9514
Alcohol-soluble	6.4699	5.3123	0.4169
Alcohol-insoluble	2.6990	1.7812	0.3485
Residue	0.8177	2.6661	0.1860

The viscous opalescent supernatant of the pepsin digestion (P-Sup, Fig. 2) was precipitated at room temperature by the dropwise addition of ethyl alcohol to a final concentration of 80 per cent. The alcohol was added to the supernatant while the latter was under gentle mixing by a magnetic stirrer. The resulting precipitate was white, stringy, and rubbery, resembling in this property the precipitate originally obtained by addition of alcohol to whole sputum. The precipitate was successively washed with 95 per cent ethanol, absolute ethanol, and finally with ether, after which it was dried in vacuum (PEtOH); the yield of this fraction was 2.6990 Gm., representing 27 per cent of the starting material.

To 1.0015 Gm. of PEtOH was added 10.0 ml. of 90 per cent (w/v) aqueous phenol; the mixture was held at room temperature for twenty-four hours, during which time the flask containing the mixture was shaken vigorously at frequent intervals. After twenty-four hours, the mixture was centrifuged at 16,000 r.p.m. for twenty minutes at room temperature. The supernatant was decanted and the residue was re-extracted twice in the same fashion.

The combined supernatants were recentrifuged to eliminate all insoluble material and the resulting viscous supernatant (PPS) was precipitated, with the aid of the magnetic stirrer, by adding a 1:1 mixture of 90 per cent phenol and absolute alcohol to a final alcohol concentration of 30 per cent, at which concentration maximal precipitation of this fraction occurred. This precipitate was completely water soluble, whereas precipitates at higher concentrations of alcohol were incompletely soluble. This phenol-soluble fraction, insoluble in 30 per cent alcohol, represented 2.0 per cent of starting material CI and is labeled PPS-30 in Fig. 2.

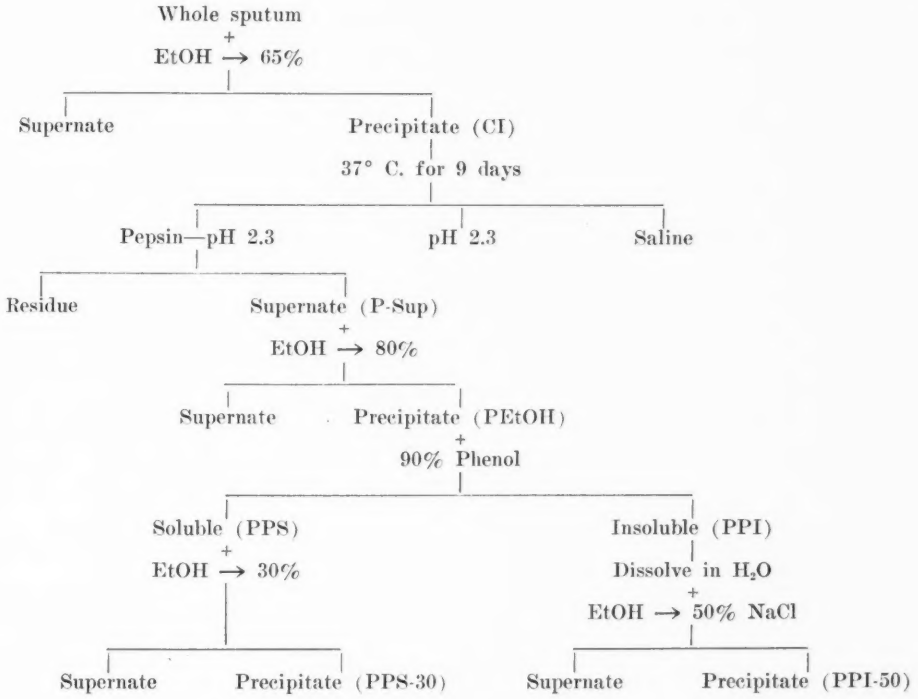

Fig. 2.—Scheme of extraction of whole asthmatic sputum.

The phenol-insoluble residue (PPI), which comprised 17.8 per cent of the starting CI, was apparently completely soluble at 2 per cent concentration in distilled water without adjustment of pH. A small amount of insoluble material which sedimented on tenfold dilution of the 2 per cent solution was removed by centrifugation. The highly viscous 2 per cent aqueous solution of PPI was then precipitated by the dropwise addition of absolute ethanol to a final concentration of 50 per cent. Precipitation occurred only after the addition of 19.5 per cent (v/v) of a saturated solution of sodium chloride in the manner employed by Gibbons and Morgan[45] in the purification of blood group substance from a pseudomucinous cyst fluid derived from a group B subject. A copious white, sticky precipitate (PPI-50) was then obtained, representing

5 per cent of the starting material (CI). Upon centrifugation, a gelatinous button was obtained; this was dialyzed against distilled water for seven hours at 4° C., with hourly changes of water and inversion every fifteen minutes, and then overnight against the final change of water. The gelatinous dialyzed residue was lyophilized; it yielded a pure white, sticky powder which was kept under vacuum. This material was completely soluble in water and was highly viscous.

The scheme of this extraction is summarized in the flow diagram (Fig. 2). Identical extractions were performed at an acid pH and at neutral pH without addition of pepsin. These yielded lesser amounts of starting material in the supernatants than was obtained from the pepsin digest (Table I).

C. *Analytical Procedures Applied to Sputum Fractions.*—

1. The determinations of total nitrogen, total carbohydrate, and hexosamine were made by the methods already described.[36-40]

2. Fucose determinations were made by the method of Dische and Shettles[46] and Werner,[33] and per cent n-acetylhexosamine determinations were made by the method of Aminoff, Morgan, and Watkins.[47]

3. Relative viscosities[48] of the sputum fractions were ascertained with the aid of a capillary viscosimeter (Ostwald-Fenske)[49a and b] at a temperature of 25° C. ± 0.5° C.

4. Hemagglutination inhibition was by the method of Baer, Bringaze, and McNamee.[50] In this procedure 0.1 c.c. of varying dilutions of antigen were incubated in 10 by 75 mm. tubes at 37° C. for thirty minutes with two hemagglutinating units of isoagglutinin contained in an 0.2 c.c. volume. To the incubated mixture, 0.1 c.c. of a 4 per cent suspension of erythrocytes was added; the mixture was shaken and incubated for thirty minutes at 37° C., after which all tubes were centrifuged for three minutes at 1,000 r.p.m. in a Size 1 International centrifuge equipped with a horizontal head. The degree of hemagglutination was estimated by direct visualization in the usual way.

5. Free electrophoresis of the PPS-30, PPI-50, and commercial A and B substances (Fig. 6) was carried out on a Perkin-Elmer apparatus. All samples were in a concentration of 0.5 per cent by dry weight in 0.15 M sodium chloride and 0.02 M phosphate buffer pH 7.4. All runs were at 0.012 Ma. with a resistance of 34 ohms; A and B substances were run for 7,980 seconds, PPS-30 for 16,700 seconds, and PPI-50 for 6,240 seconds.

RESULTS AND DISCUSSION

The chemical data obtained on analysis of the first nineteen ethanol precipitates of sputa are given in Table II. There is considerable spread in the range of all of the analytical procedures applied, suggesting that the single maneuver of ethanol precipitation was insufficient to accomplish more than a very crude purification. The sputum containing 13.3 mg. per milliliter of total solids was the most homogeneous and yet was among the most viscous of all the sputa, indicating that the viscosity of sputum was not dependent on the

TABLE II. RANGE OF CONSTITUENTS IN NINETEEN EtOH PRECIPITATED ASTHMATIC SPUTA

Total solids—13.3 to 33.8 mg./ml.
65 per cent EtOH insoluble—6.0 to 19.9 mg./ml.
Total carbohydrate as glucose—6.2 to 28.2 per cent
Hexosamine—11.9 to 15.0 per cent
Total nitrogen—9.0 to 13.0 per cent

concentration of *all* the solids within it. The chemical data differ from those of pure blood group substance in that the crude ethanol precipitates were considerably higher in nitrogen and lower in total carbohydrate and hexosamine.

Fig. 3.—Two-dimensional paper chromatogram of the 6N hydrochloric acid hydrolysate of the alcohol-insoluble fraction of sputum No. 3. Stained with ninhydrin. *A*, Cystine; *B*, aspartic acid; *C*, glutamic acid; *D*, glycine; *E*, serine; *F*, threonine; *G*, alanine; *H*, hydroxyproline; *I*, histidine; *J*, gamma-aminobutyric acid; *K*, proline; *L*, methionine; *M*, valine; *N*, leucine; *O*, tyrosine; *P*, arginine; *Q*, lysine; *R*, over-tyrosine; *S*, beta-aminoisobutyric acid; *T*, hexosamine; *U*, taurine; *V*, phenylalanine; *W*, beta-alanine; *X*, alpha-amino-n-butyric acid.

Two-dimensional chromatograms (phenol-lutidine) of eighteen-hour 6N hydrochloric acid hydrolysates of the alcohol precipitates failed to yield evidence of distinctive differences among the various sputa, and the amino acid patterns were those typically observed with proteins. The following amino acids were identified: cystine, aspartic acid, glutamic acid, glycine, serine, threonine, alanine, histidine, proline, methionine, valine, leucine, phenylalanine, tyrosine, over-tyrosine, arginine, lysine, hexosamine, and taurine (Fig. 3). Of some interest is the fact that all the hydrolysates yielded higher concentrations of threonine and phenylalanine than are encountered with similar chromatograms of the usual protein hydrolysates. This finding is in agreement with

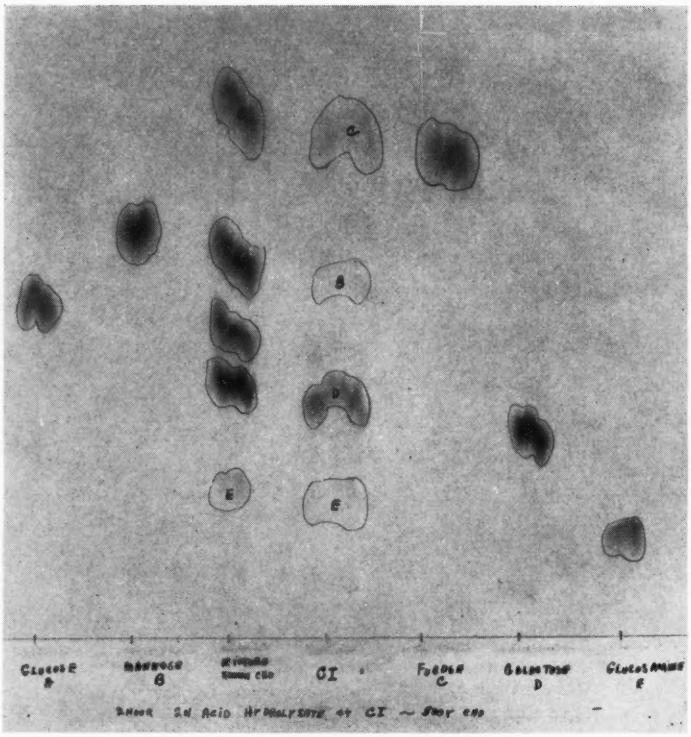

Fig. 4.—One-dimensional chromatogram (pyridine-amyl alcohol-water) of 2N sulfuric acid hydrolysate of CI, stained with aniline hydrogen phthalate.

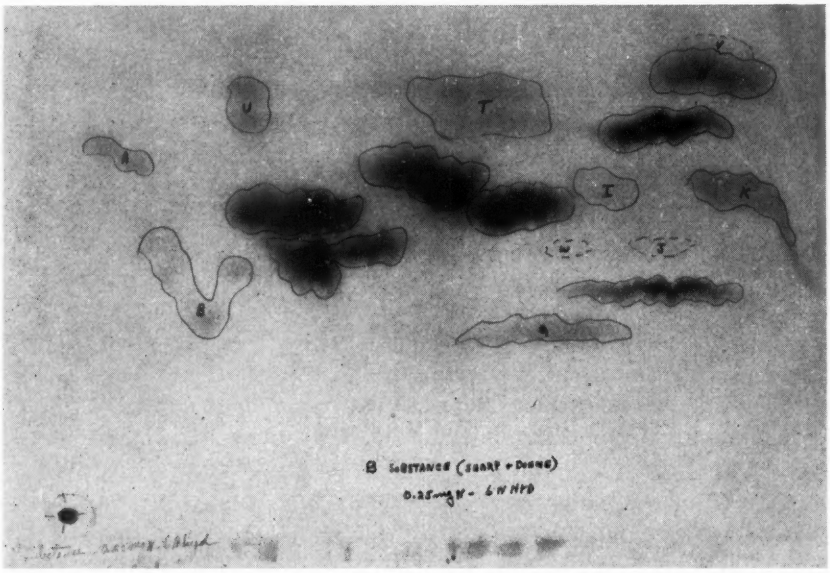

Fig. 5.—Note absence of phenylalanine (*V*) and tyrosine (*O*) and over-tyrosine (*R*) from this two-dimensional chromatogram of purified B substance (Merck Sharp & Dohme).

Morgan's[51] observations with human A, B, and O blood group substances. The one-dimensional chromatograms of two-hour 2N sulfuric acid hydrolysates prepared for carbohydrate analysis showed the presence of galactose, mannose, fucose, and hexosamine, the latter not yet having been further identified (Fig. 4). This reveals the same individual sugars found in hydrolysates of blood group substance.

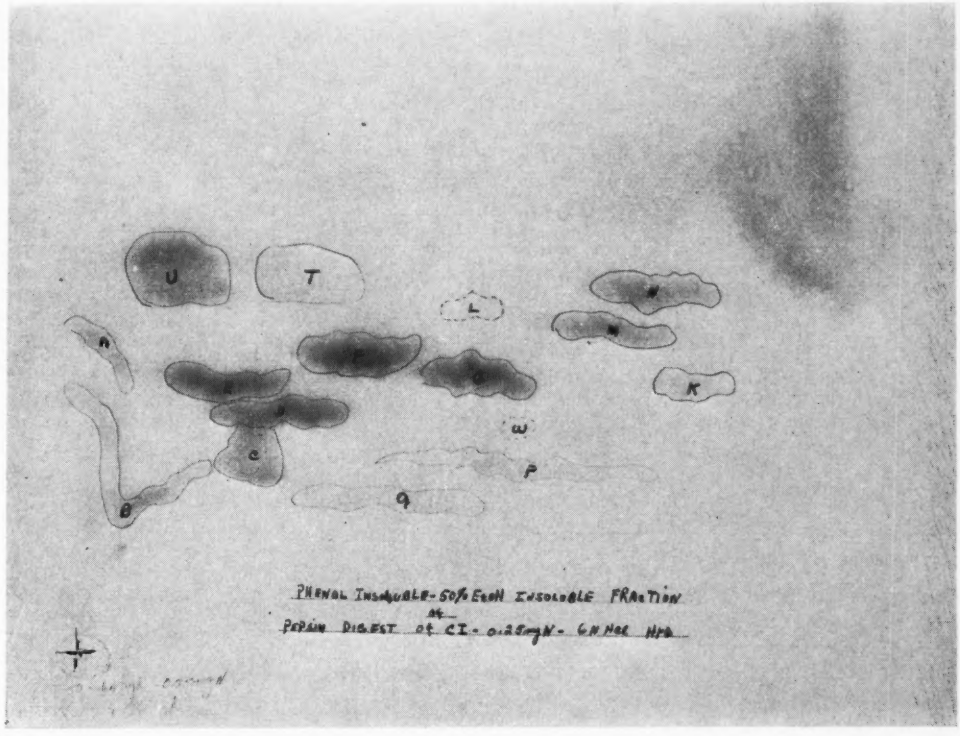

Fig. 6.—Same as Fig. 5, but hydrolysate of PPI-50.

The results of chromatographic analysis also indicate that non-blood-group-substance protein is present in the ethanol precipitates. This is based on the demonstration of considerable amounts of the aromatic amino acids (phenylalanine and tyrosine) in the hydrolysates of sputum (Fig. 3) and the relative absence of these from similar hydrolysates of purified blood group substance (Fig. 5) (see also Fig. 6, which reveals absence of the aromatic amino acids from a hydrolysate of PPI-50).

The alcohol-insoluble residues failed to dissolve readily when rehydrated, and there was considerable variation in the degree of viscosity which developed after rehydration. It was for this reason that additional fractionation was attempted and the pepsin digestion-phenol extraction method was selected, since it was evident from the analysis of the ethanol precipitates that a material similar to blood group substance must be present in large amounts, a fact which

Werner[33] had already established as characteristic of all epithelial mucus. Since Morgan[52, 53] had used phenol very effectively for the isolation of blood group substance from mucosa, saliva, and cyst fluids, this was carried out first with an ethanol precipitate. The low yield of a relatively nonviscous product prompted application of pepsin digestion after the method of Bendich, Kabat, and Bezer[44] and, as indicated, it gave a viscous water-soluble, phenol-insoluble fraction in approximately a 5 per cent yield (PPI-50). The yield of this material, on a weight basis, was not appreciably greater than that obtained by direct phenol extraction alone. The product obtained after pepsin digestion (PPI-50) was lower in nitrogen and higher in total carbohydrate, hexosamine, fucose, and n-acetylhexosamine, as indicated in Table III, which summarizes

TABLE III. SUMMARY OF CHEMICAL DATA OF SPUTUM FRACTIONS

SAMPLE	PER CENT TN	PER CENT TC	PER CENT HEXOSAMINE	RATIO HEXA-TC	PER CENT FUCOSE	PER CENT N-ACETYL	RELATIVE VISCOSITY
CI	9.8	16.9	8.3	0.49	5.14	3.7	—
PEtOH	6.5	37.1	18.0	0.49	10.0	7.5	—
PPS-30	8.0	31.3	18.0	0.58	6.2	8.4	1.53
PPI	3.9	31.7	20.9	0.66	10.7	8.9	1.41
PPI-50	5.3	39.5	22.3	0.57	16.4	10.8	2.69
(1)	6.4	43.5	28.1	0.65	5.8	14.1	—
A (2)	5.9 to 6.4	55 to 61	30 to 34	0.54 to 0.60		9.1 to 11.3	1.39 to 1.71
(3)	5.7 to 6.4	42 to 52	24.0 to 27.3			9.6 to 10.7	2.80 to 2.86
B (1)	6.2	33.4	23.8	0.71	6.5	12.5	
(3)	4.8 to 4.9	53.9 to 55.1	20.3 to 22.7	0.38 to 0.41	19.7 to 20.2		
O (2)	5.9 to 6.1	56 to 59	32 to 34	0.55 to 0.57		9.3 to 10.2	1.47 to 1.63

(1) = Washington University analysis of Sharp & Dohme A and B substances.
(2) = Kabat's analysis of purified A and O substances.
(3) = Morgan's analysis of purified A and B substances.

the chemical data obtained on analysis of all the fractions of sputum. It should be noted that only the phenol-insoluble fractions (PPI and PPI-50), and especially the 50 per cent alcohol-insoluble portion (PPI-50), yield data comparable to that for blood group substance. Furthermore, PPI-50 was the most viscous fraction we had obtained. The ratio of hexosamine to total carbohydrate was little affected by the purifying procedures, suggesting that the carbohydrate moiety of the original ethanol precipitate passed through the purification procedures unchanged.

Keckwick[54] and Caspary[55] had established that purified blood group substance is electrophoretically homogeneous, and for this reason the sputum fractions were studied electrophoretically. First studies were by paper electrophoresis[56] and an electropherogram of purified commercial A and B substances, the combined ethanol precipitate of sputum, and the pepsin digest-phenol fractions (Fig. 7) revealed immobility of the sputum fractions and a slow migration of A and B substances toward the cathode in Veronal buffer and toward the anode in borate buffer. This electropherogram was stained by the periodic acid-Schiff method according to the method of Koiw and Gronwall[57] as modified by Koiw,[58] and it is evident that all the sputum fractions yielded a good reaction for mucopolysaccharides.

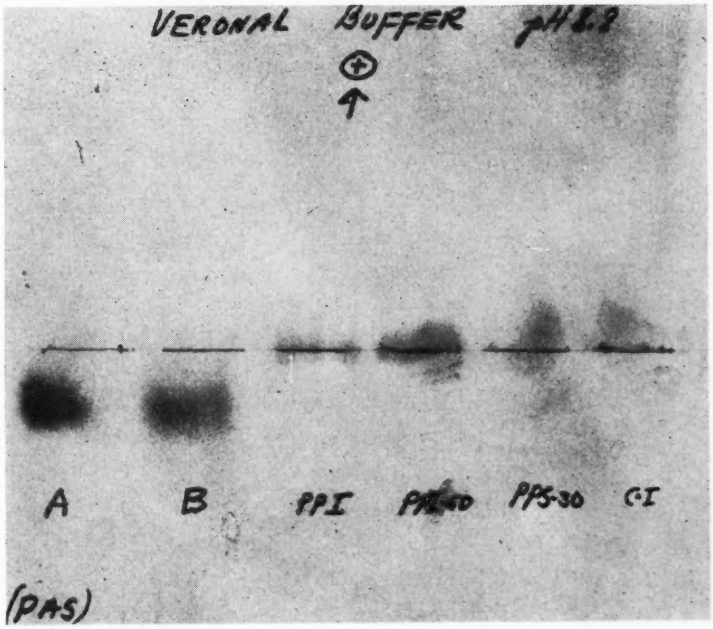

Fig. 7.—Paper electropherogram of A and B blood group substances and sputum fractions, stained with periodic acid-Schiff reagent. Run for six hours at 350 volts and 4 to 5 Ma.

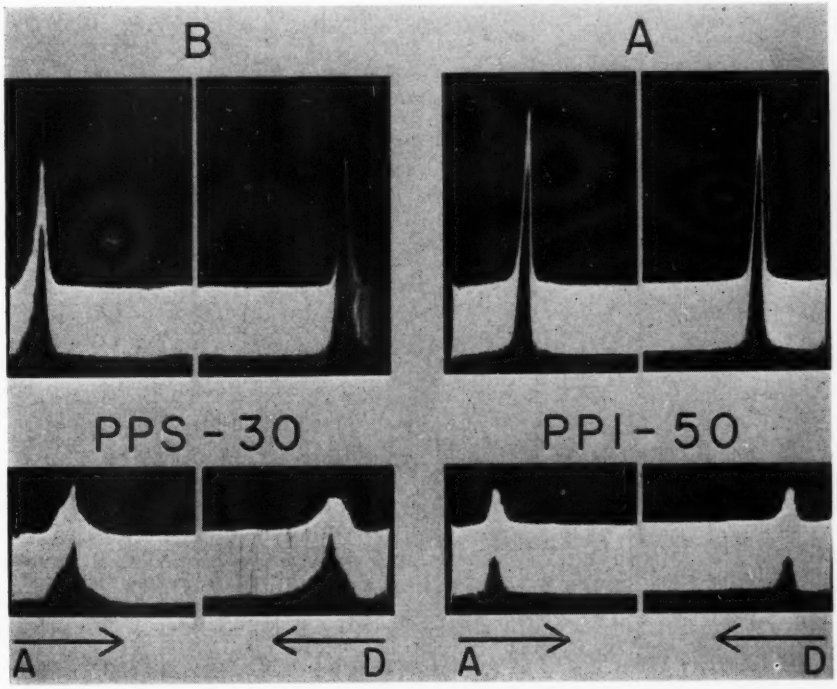

Fig. 8.—Free electrophoresis of commercial A and B substance PPS-30 and PPI-50: mobilities, respectively, -1.51×10^{-5}, -1.14×10^{-5}, -1.37×10^{-5}, and -0.28×10^{-5} cm./sec./volt.

TABLE IV. DETERMINATION OF BLOOD GROUP ACTIVITY OF SPUTUM FRACTIONS BY INHIBITION OF HEMAGGLUTINATION OF A AND B CELLS*

	A CELLS†													B CELLS†											
MCG. ADDED	100	50	25	10	5	1	0.5	0.1	0.05	0.01	0.005	0.0005	0.00005	100	50	25	10	5	1	0.5	0.1	0.05	0.01	0.005	0.0005	0.00005
A substance‡	–	–	–	–	–	–	–	–	–	–	1+	2+	3+	3+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
B substance‡	–	–	–	–	–	–	–	–	–	1+	2+	2+	3+	–	–	–	–	–	–	1+	1+	2+	2+	3+	3+	3+
PPI	–	–	–	–	–	–	–	–	–	–	1+	2+	3+	–	–	–	–	–	–	–	1+	1+	2+	3+	3+	3+
PPI-50	–	–	–	–	–	–	–	–	–	–	–	2+	3+	–	–	–	–	–	–	–	1+	1+	2+	3+	3+	3+
PPS-30	–	–	–	–	–	–	–	–	–	–	1+	2+	3+	–	–	–	–	–	–	1+	1+	1+	2+	3+	3+	3+
PEtOH	–	–	–	–	–	–	–	–	–	–	1+	2+	3+	–	–	–	–	–	–	–	–	–	–	–	1+	2+
CI	–	–	–	–	–	–	–	–	–	–	–	2+	2+	–	–	1+	1+	2+	2+	2+	2+	3+	3+	3+	3+	3+
Ragweed pollen§	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	–	–	–	2+	2+	2+	2+	3+	3+	3+	3+	3+	4+
Saline	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+

*Commercial human blood-typing sera (Michael Reese); 0.1 ml. antigen, plus 0.2 ml. serum dilution, plus 0.1 ml. 4 per cent RBC.
†–, 1+, 2+, 3+, 4+ denote complete, strong, moderate, slight, and no inhibition, respectively.
‡A and B blood group substances kindly furnished by Dr. R. T. Smith, Sharp & Dohme.
§Quantities of extract designated as protein nitrogen units.

The failure to obtain migration of sputum fractions on paper prompted an examination of fractions PPS-30 and PPI-50 by free electrophoresis. The result of one such study is illustrated in Fig. 8, which reveals distinct differences in the mobility and peak characteristics of commercial A (hog) and B (horse) purified blood group substances as compared with the sputum fractions. The sputum fractions, however, did exhibit mobility on free electrophoresis, in contrast to their immobility on paper.

The data obtained on electrophoresis suggested that there were differences between the blood group substance of commercial origin and that derived from asthmatic sputum. The PPI-50 derived from sputum, although very active in hemagglutination inhibition (Table IV), was nevertheless devoid of antigenicity when injected intravenously into rabbits.[59] In this respect it resembled purified commercial blood group substance and differed from whole sputum, which was quite antigenic.[59]

SUMMARY

1. The significance in asthma of intrabronchial accumulation of a viscid mucus and previous studies on its chemical nature are briefly reviewed.

2. The present article describes the immunochemical methods applied to the analysis of a pooled ethanol precipitate of twenty-eight sputa obtained from asthmatic patients.

3. Confirming observations on epithelial mucus by other investigators, we found that asthmatic sputum contained hexosamine, n-acetylhexosamine, and fucose and that it was devoid of uronic acids and nucleoprotein. A major portion of the material appeared to be a carbohydrate linked to a protein having the characteristics of a mucopolysaccharide.

4. Fractionating procedures successful in isolating blood group substance were applied to the ethanol precipitates of sputum. An approximately 5 per cent yield of mucopolysaccharide closely resembling blood group substance chemically and possessing considerable blood group activity in hemagglutination inhibition studies was obtained.

5. The isolated mucopolysaccharides were soluble in water, they were viscous, and they differed electrophoretically from commercial A and B substances.

6. The studies to date point up the desirability of extending the observations by analyzing individual sputa from asthamtic subjects of known blood groups.

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STUDIES OF MATERIALS DERIVED FROM STAPHYLOCOCCUS

IV. Precipitating Antibodies to Staphylococcal Antigens in Human Sera

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IT HAS been suggested that hypersensitivity to bacteria may be an important factor in some allergic patients. Because of their ubiquity, staphylococci have been foremost among the suspects. In studies of cutaneous reactions to polysaccharide fractions prepared from staphylococcal cell extracts, it was found that a group of hypersensitive persons had a higher percentage of positive reactors than a group of normal persons.[1] Protein fractions from these same extracts were found to be anaphylactogenic in guinea pigs.[2, 3] In this communication we shall report the result of an investigation of the in vitro reaction between human sera and staphylococcal antigens, using an agar precipitin technique.[4]

METHODS AND MATERIALS

Three strains of staphylococci were used. F-10 and F-7 have been described previously.[1] F-10 is a typical *Staphylococcus aureus,* while F-7 is a *Staphylococcus albus.* Wood 46 (W-46) strain, which was obtained from Lederle Laboratories, is a *Staphylococcus aureus,* a potent producer of α-toxin; it is used commercially for the preparation of staphylococcal toxin and toxoid.

Bacterial extracts were prepared from washed, dried bacterial cells, as previously described.[1] The supernatant fluid, cleared of bacteria by centrifugation, was termed "culture filtrate." Approximately tenfold concentrates of these preparations were obtained either by precipitation with ammonium sulfate at 75 per cent of saturation followed by resolution in saline or by evaporation from a dialysis sac in a stream of air. The concentrates were dialyzed overnight against physiologic saline at least ten times. All preparations were stored in the refrigerator until used.

The Ouchterlony agar precipitin method,[4] as modified by Halbert and associates,[5] was used. In this modification Veronal (sodium barbital) is incorporated in the agar and the pH is carefully adjusted to 7.5 to reduce the development of opacity around serum wells.

Human gamma globulin (HGG), prepared from pooled human sera, was obtained from Lederle Laboratories, Sharp & Dohme, Pitman-Moore Company,

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and the American Red Cross. These were stored in the refrigerator at about 4° C. Individual human sera were stored at –20° C.

RESULTS

The result obtained when gamma globulin was placed in the center well and culture filtrates of F-10, F-7, and W-46 were placed in the surrounding wells is shown in Fig. 1. The position of a band between two wells depends on the initial concentrations of the antigen and the antibody which form the band and on their diffusion coefficients. If an antigen is present in very high or very low concentration, its band may be missed, as its reaction with antibody

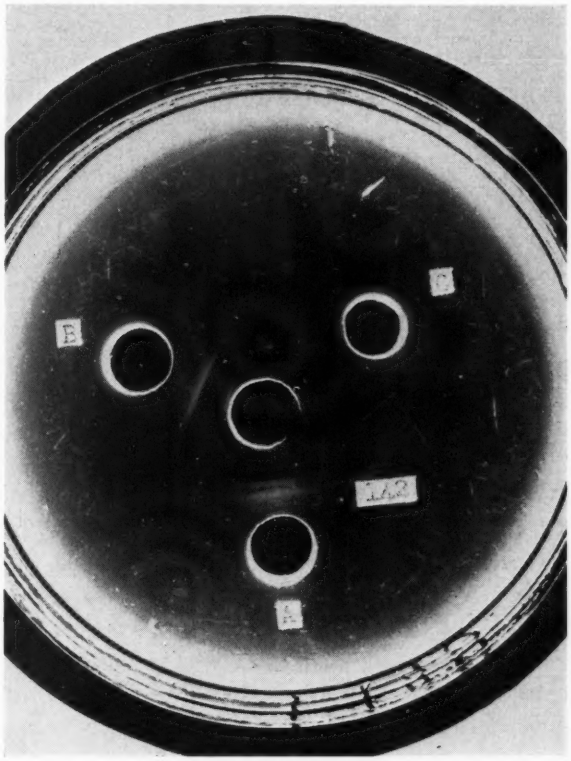

Fig. 1.—Reaction of staphylococcal culture filtrates with pooled human gamma globulin. Gamma globulin (Pitman-Moore) in center well. *A*, F-10 culture filtrate; *B*, W-46 culture filtrate; *C*, F-7 culture filtrate.

may then occur too close to, or actually within, the antibody or antigen well. Therefore, various concentrations of the extracts and culture filtrates were tested to detect the maximum possible number of bands.

Multiple bands appeared between each of the bacterial preparations from the aureus strains and HGG (Table I). No bands were formed between extract or culture filtrate of F-7 (albus) and HGG, and in control studies no bands were formed between the media used and HGG. Essentially identical results were obtained with all of the HGG preparations studied except the material from

TABLE I. BANDS OBTAINED BETWEEN HUMAN GAMMA GLOBULIN AND STAPHYLOCOCCAL EXTRACTS AND CULTURE FILTRATES

PREPARATION	NUMBER OF BANDS
F-10 filtrate	8
F-10 extract	2
W-46 filtrate	5
W-46 extract	3
F-7 filtrate	0
F-7 extract	0

Sharp & Dohme, which showed fewer bands. Most of the antibodies were directed toward antigens present in the culture filtrate rather than toward those in the bacterial extracts.

DISCUSSION

Cutaneous reactions to materials prepared from staphylococcal bacterial extracts have been previously reported.[1] Culture filtrates were not similarly studied. The present study indicates that antigens present in the culture filtrate should be considered possible factors in hypersensitivity. In fact, HGG contains antibodies directed toward a greater number of antigens in the culture filtrate than the bacterial extracts.

Halbert and associates[5] have reported similar findings in their investigations of human streptococcal infections. The antibodies in human sera that were directed toward "extracellular" antigens of the streptococcus were more numerous than those directed toward cellular antigens (bacterial extracts). Halbert and associates also emphasize one of the main advantages of the agar precipitin technique, namely, that it makes possible estimation of a number of distinct antibody responses of the human being to various infectious agents.

One may question the implication of these studies in the field of bacterial allergy. Precipitating antibodies are under investigation here, while the nature of the skin-sensitizing antibody (reagin) is unknown. Human sera from several ragweed-sensitive patients, both treated and untreated, failed to form bands with ragweed extract.[6] However, Kuhns[7] has found that skin-sensitizing antibody to diphtheria toxin coprecipitates with precipitating antibody to the toxin. This suggests that precipitating and skin-sensitizing antibodies may be formed to the same antigen. Since an in vitro method for analyzing skin-sensitizing antibodies has yet to be developed, investigation of precipitating antibodies by the method used here appears to offer the most suitable in vitro means for determining the antigens which may be involved in sensitization to infectious agents.

Another point of interest is the absence of bands between the samples of pooled human gamma globulin and F-7, a strain of *Staphylococcus albus,* whereas numerous bands were formed with the two strains of *Staphylococcus aureus.* Preliminary studies with additional strains of *Staphylococcus aureus* and *Staphylococcus albus* have shown that pooled human gamma globulin contains antibodies to all of the twenty-two aureus strains studied, while only two of nine albus strains formed bands with the gamma globulin preparations. These

observations suggest that invasiveness or pathogenicity of a strain of staphylococcus may play a role in its ability to sensitize human beings. However, definite conclusions will require further investigation.

SUMMARY

Through use of the agar precipitin method, human sera have been shown to contain antibodies directed against both culture filtrates and bacterial extracts of staphylococci. Most of the antibodies were directed against culture filtrates. All of the *Staphylococcus aureus* strains studied produced antigens which reacted with HGG, while only two of nine *Staphylococcus albus* strains formed antigens reacting with HGG. This suggests a relationship between the pathogenicity of a strain of staphylococcus and its ability to stimulate antibody production.

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STUDIES OF MATERIALS DERIVED FROM STAPHYLOCOCCUS

V. Immunologic Relationship Between a Nucleoprotein Fraction of Two Strains of Staphylococcus as Determined by Anaphylaxis

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IN PREVIOUS studies from this laboratory a crude nucleoprotein fraction, precipitated at pH 3.5 (fraction A), was obtained from saline extracts of ground, washed cells of micrococcus (staphylococcus) organisms.[1] Anaphylaxis to fraction A from *Micrococcus (Staphylococcus) albus* could be produced in isolated segments of ileum from guinea pigs sensitized to this fraction. However, anaphylaxis could not be demonstrated with fraction A obtained from hemolytic *Staphylococcus aureus* strains, for these fractions had a primary anaphylactoid effect, indistinguishable from anaphylaxis, upon the isolated ileum of normal guinea pigs.[2, 3]

It was subsequently found that gamma globulin from a variety of animal species protected the isolated guinea pig ileum against the anaphylactoid effect of fraction A derived from hemolytic *Staphylococcus aureus* (F-10), but did not inhibit anaphylaxis.[3] Therefore, it was postulated that it should be possible to demonstrate anaphylaxis to fraction A from *Staphylococcus aureus,* despite its primary toxicity, by prior addition of gamma globulin to the bath containing the isolated, suspended segment of ileum from a sensitized guinea pig. The gamma globulin should protect the ileum segment against the toxic or anaphylactoid effect of fraction A but allow the anaphylactic reaction to occur. If anaphylaxis could be so demonstrated, it would then be possible to investigate the allergenic relationship between fraction A of hemolytic *Staphylococcus aureus* and fraction A of *Staphylococcus albus.*

In the present communication the results of experiments designed to test this hypothesis are reported.

MATERIALS AND METHODS

Fractions A (F-10) and A (F-7) are derived from a hemolytic *Staphylococcus aureus* strain (F-10) and a *Staphylococcus albus* strain (F-7), respectively, as previously reported. Sensitization was attempted in twenty-nine guinea pigs, each weighing 250 to 300 grams (Table I). Fifteen animals were initially injected with fraction A (F-10) and fourteen with A (F-7) for four consecutive

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days. Because preliminary studies had demonstrated that sensitization was successful only when booster injections were given, all animals in both groups received four boosters on the eighteenth, fiftieth, sixty-fourth, and ninety-ninth days, respectively, after initial immunization. One animal from each group was challenged periodically with the homologous fraction until the last remaining pair of animals, one from each group, was challenged 117 days after initial sensitization or eighteen days after the last booster.

TABLE I. SCHEDULE OF EXPERIMENTAL PROCEDURES AND RESULTS OF TESTS FOR ANAPHYLAXIS

DAY	ANAPHYLAXIS		INJECTION SCHEDULE
	A(F-10)	A(F-7)	
1			
2			15 A (F-10) animals and 14 A (F-7) animals given 0.1 mg. protein nitrogen subcutaneously per day
3			
4			
18			29 animals: 0.01 mg. protein nitrogen intradermally
47	Neg.	Pos.	
50			27 animals remaining: 0.05 mg. protein nitrogen subcutaneously
61	Neg.	Pos.	
64			25 animals remaining: 0.05 mg. protein nitrogen subcutaneously
75	Neg.		
79	Neg.		
86		Pos.	
89	Pos.	Pos.	
93	Neg.	Pos.	
94	Neg.	Pos.	
96	Neg.	±	
99			7 F-10 animals remaining: 0.02 mg. protein nitrogen subcutaneously 7 F-7 animals remaining: 0.05 mg. protein nitrogen subcutaneously
100	±	Neg.	
101	Neg.	Pos.	
103	Neg.	Pos.	
106	Pos.	Pos.	
108	Neg.	Pos.	
110	Pos.	Pos.	
117	Neg.	Pos.	

Anaphylaxis in the guinea pigs was tested with 1- to 2-inch freshly isolated segments of ileum, suspended in a 5 ml. bath containing Tyrode's solution at 37° C. The upper end of the segment was tied to a lever which recorded longitudinal movements of the segment on a smoked drum rotating at a speed of 2.5 cm. per minute.

In segments from animals being tested for anaphylaxis to fraction A (F-10), 8.0 mg. of pooled human gamma globulin was first added to the bath, and after approximately 30 seconds 0.1 to 0.2 mg. protein nitrogen of A (F-10) was added to the bath. If contraction occurred in the presence of gamma globulin, anaphylaxis was considered to have occurred. The record was then continued until the segment relaxed, and the bath was washed three times. The process was repeated; gamma globulin was again added, followed by fraction A, and if no reaction occurred on this occasion desensitization was considered to have been demonstrated. Histamine was then added to the bath five times, with three

washes after each histamine-induced contraction, in order to remove all of the gamma globulin; this was followed by a third and fourth addition of fraction A (F-10), which was expected to produce an anaphylactoid reaction followed by tachyphylaxis, as demonstrated in previous studies.[2]

In previous studies gamma globulin consistently prevented the anaphylactoid contraction of the isolated guinea pig ileum to fraction A (F-10).[3] Therefore, if contraction occurred upon addition of A (F-10) in the presence of gamma globulin and if the second addition of gamma globulin and fraction A resulted in no contraction, an anaphylactic reaction followed by desensitization was considered to have occurred. As further proof that the reaction was anaphylactic, to be distinguished from the primary anaphylactoid effect of this fraction, it was necessary to show that, following desensitization and removal of gamma globulin from the bath, A (F-10) produced the typical anaphylactoid reaction, followed by tachyphylaxis.

Although it was unnecessary to use gamma globulin in the anaphylaxis studies with A (F-7), since this fraction has no primary toxicity, gamma globulin was placed in the bath prior to the A (F-7) in most experiments in order to maintain identical conditions in both groups.

Since fraction A (F-7) is nontoxic, the criteria for anaphylaxis were simpler than those for A (F-10), consisting merely of a contraction of the ileum segment following the initial addition of A (F-7) with loss of reactivity to this fraction upon subsequent additions, that is, desensitization.

When reactions were very slight or results were difficult to evaluate for technical reasons, they were labelled "indeterminate" or "plus-minus," but were considered negative for anaphylaxis in the final tabulation.

One to four segments from each animal were studied, and if any segment demonstrated anaphylaxis the animal was considered positive.

Cross-anaphylaxis and cross-desensitization studies were carried out by challenging segments from animals sensitive to either of the fractions with the other. Addition to the bath of A (F-10) was always preceded by addition of gamma globulin. When a cross reaction occurred, the segment was desensitized to the cross-reacting fraction by repeated additions, until no further reaction occurred, and then the segment was challenged with the homologous fraction. If no reaction or only a slight reaction occurred with this final challenge, cross desensitization was considered complete or partial, respectively.

RESULTS

Using the above-mentioned criteria, anaphylaxis was demonstrated in four of the fifteen guinea pigs injected with fraction A (F-10) and in twelve of the fourteen guinea pigs in the A (F-7) group (Table I, Figs. 1 and 2). Two of the positively reacting animals in the F-10 group appeared fifteen and twenty-five days after the third booster injection and the other two appeared seven and eleven days after the fourth booster injection.

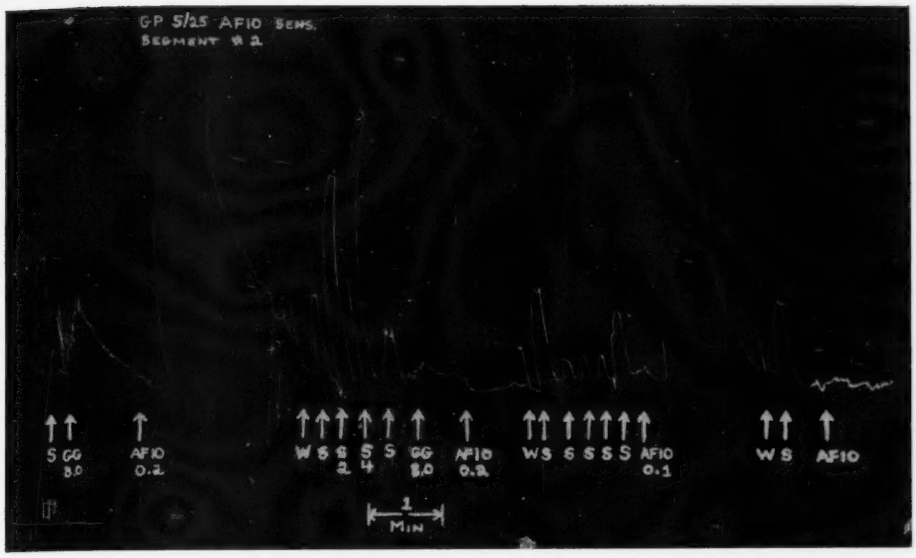

Fig. 1.—Anaphylaxis to hemolytic *Staphylococcus aureus* fraction A (F-10), as tested on isolated ileum of guinea pig. *S* indicates addition of 0.1 mcg. of standard histamine base, followed by three washes at height of contraction. *W* indicates three washes.

At *GG*, 8.0 mg. protein nitrogen of pooled human gamma globulin is added to bath. After one minute addition of 0.2 mg. protein nitrogen of A(F-10) is followed by maximal contraction (anaphylaxis).

After washing (*W*) and addition to the bath of histamine in doses of 0.1 (*S*), 0.2 (2*S*), 0.4 (4*S*), and 0.1 mcg., respectively, with three washes after each dose, the second addition of 0.2 mg. protein nitrogen of A(F-10) in the presence of 8.0 mg. gamma globulin results in no reaction (desensitization to A[F-10]).

After five additions of histamine standard (*S*), with three washes after each to remove gamma globulin, addition of 0.1 mg. protein nitrogen of A(F-10) results in maximal contraction (anaphylactoid reaction). After washing and histamine standard (*S*), final addition of 0.1 mg. protein nitrogen of A(F-10) is without effect (tachyphylaxis).

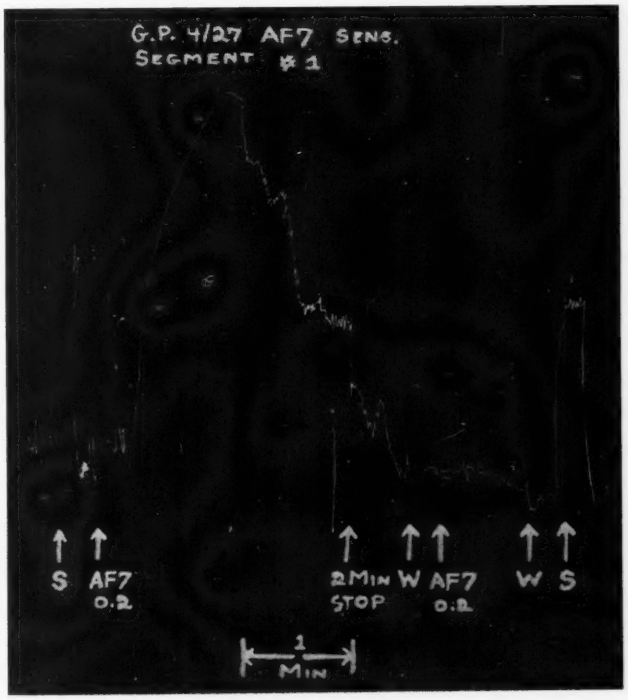

Fig. 2.—Anaphylaxis to *Staphylococcus albus* fraction A (F-7), as tested on isolated ileum of guinea pig. *S* and *W* as in Fig. 1.

Note that 0.2 mg. protein nitrogen of A(F-7), followed by maximum contraction, persists for several minutes (anaphylaxis). A second addition of the same fraction has no effect (desensitization).

Cross anaphylaxis was studied in the four animals sensitive to A (F-10) and in one additional A (F-10)-sensitive animal from the preliminary group, which was not otherwise included in the present data. Cross anaphylaxis to A (F-7) was demonstrated in four of these five guinea pigs (Figs. 3 and 4). In three, desensitization with the cross-reacting A (F-7) resulted in complete desensitization to the homologous A (F-10) (Fig. 4), and in the fourth cross-desensitization to the homologous A (F-10) was partial.

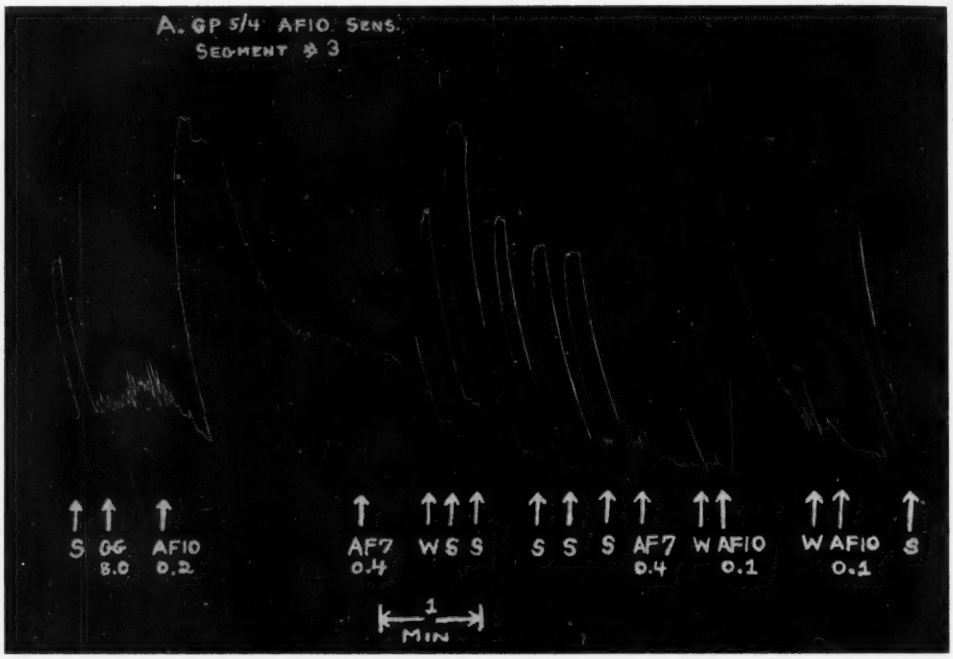

Fig. 3.—Cross anaphylaxis to A(F-7) in isolated ileum of guinea pig sensitized to A(F-10). *S* and *W* as in Fig. 1.

A. Anaphylaxis to homologous fraction A (F-10) demonstrated in manner identical with experiment shown in Fig. 1. Note that A(F-7), added after anaphylaxis to A(F-10), causes no contraction, whether added in the presence or in the absence of gamma globulin.

B, Cross anaphylaxis to heterologous fraction A (F-7) and cross desensitization to homologous A (F-10). Ileum segment adjacent to that used in Fig. 3, *A*. Initial addition of 0.4 protein nitrogen A(F-7) in presence of gamma globulin, 8.0 mg. protein nitrogen (GG 8.0), followed by contraction equivalent to more than 0.1 mcg. of histamine (*S*) (cross anaphylaxis to heterologous A[F-7]). After relaxation of segment and washing, A(F-7) in presence of gamma globulin (GG 8.0) causes no contraction (desensitization to A[F-7]). Addition of 0.2 mg. protein nitrogen of A(F-10), still in the presence of gamma globulin and F-7, causes no contraction (desensitization to homologous A[F-10]).

Finally, after five histamine additions (*S*) with three washes after each, two additions of 0.1 mg. protein nitrogen of A(F-10), in the absence of gamma globulin, show a typical anaphylactoid reaction and tachyphylaxis, respectively.

Cross anaphylaxis was studied in seven of the twelve A (F-7)-sensitive animals by challenging isolated ileum segments with A (F-10). In three of the seven, cross anaphylaxis with A (F-10) was demonstrated (Figs. 5 and 6). Cross desensitization to homologous fraction A (F-7) after desensitization with the cross-reacting fraction A (F-10) was demonstrated in two of these three animals, although the cross desensitization in both was only partial (Fig. 6).

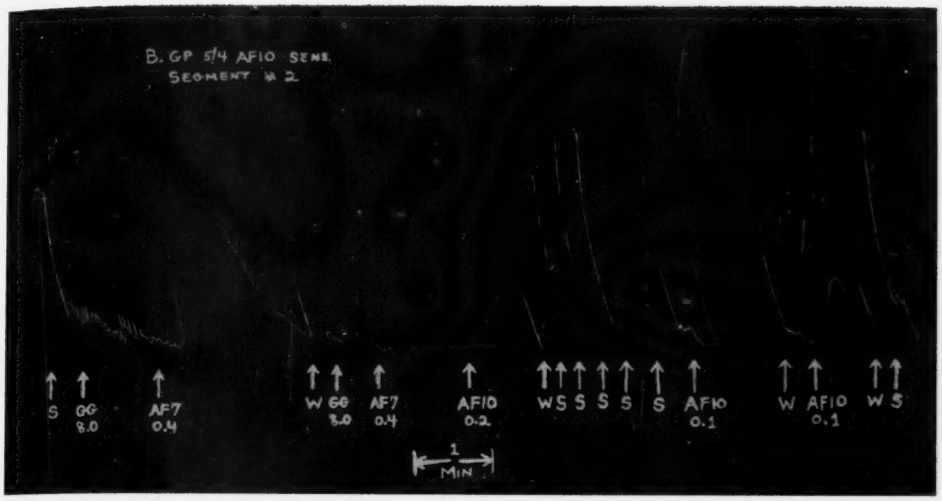

Fig. 4.—Cross anaphylaxis to A(F-7) in isolated ileum of guinea pig sensitized to A(F-7). *S* and *W* as in Fig. 1.

A, Anaphylaxis to homologous fraction A (F-7), 0.2 mg. protein nitrogen is demonstrated as in Fig. 2. Second addition of the same dose causes no reaction (desensitization).

B, Cross anaphylaxis to heterologous fraction A (F-10) with cross desensitization to homologous A (F-7). Ileum segment adjacent to that in Fig. 4, *A*. Initial addition of 0.2 mg. protein nitrogen of A(F-10) in the presence of 8.0 mg. gamma globulin (GG 8.0) is equivalent to more than 0.1 mcg. histamine (S) (cross anaphylaxis to heterologous A[F-10]). Second addition of 0.2 mg. protein nitrogen of A(F-10) in the presence of 8.0 mg. gamma globulin is followed by no reaction (desensitization to heterologous A[F-10]).

First addition of 0.2 mg. A(F-7) is followed by very slight contraction, equivalent to about one-half that produced by 0.1 mcg. histamine (*S*) (definite, but incomplete desensitization to the homologous A[F-7]). Second addition of A(F-7) is without effect (complete desensitization).

Finally, addition of A(F-10), in the absence of gamma globulin, produces the typical anaphylactoid contraction, followed by tachyphylaxis with the second addition.

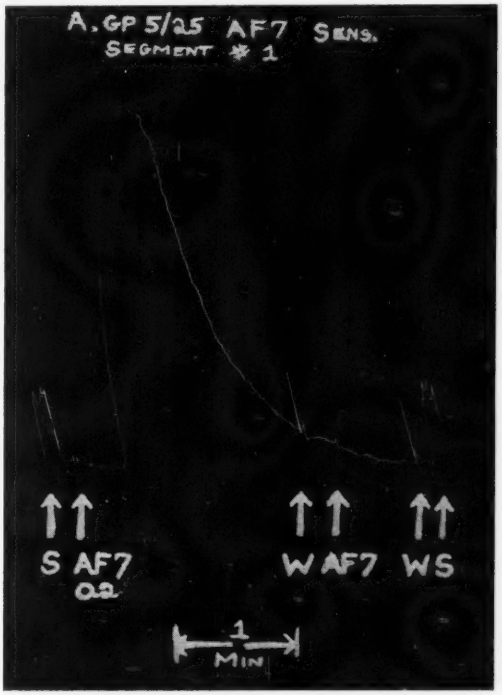

Fig. 5.

There was no example of cross desensitization which was not preceded by cross anaphylaxis. Three ileum segments from the A (F-10)-sensitized animal which failed to cross react to A (F-7) showed anaphylaxis to A (F-10). Similarly, anaphylaxis to the homologous A (F-7) was demonstrated in six segments from the four A (F-7)-sensitized animals which did not cross react with A (F-10).

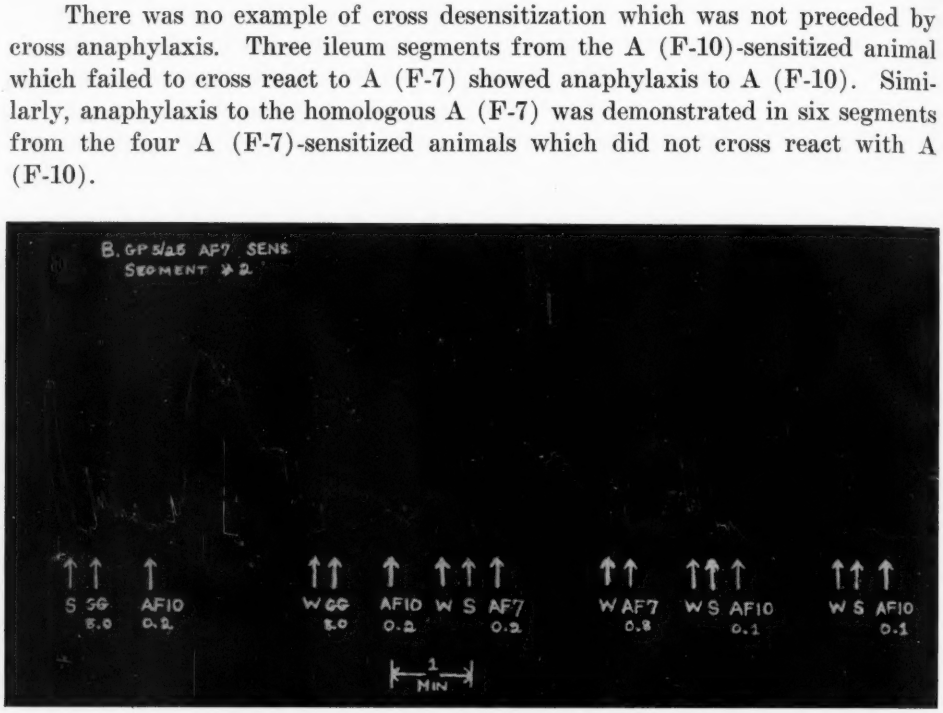

Fig. 6.

DISCUSSION

In a recent study[3] we found that gamma globulin from sera of various species prevented the anaphylactoid reaction regularly produced by a primarily nucleoprotein fraction (fraction A) derived from certain strains of hemolytic *Staphylococcus aureus.* On the other hand, gamma globulin did not have any apparent effect upon anaphylaxis, as demonstrated with the isolated ileum from guinea pigs sensitized to various proteins including egg white, streptococcus proteinase, and a simple protein fraction derived from *Staphylococcus aureus.* It was therefore postulated that this protective effect of gamma globulin against the anaphylactoid reactions produced by the toxic fraction A derived from *Staphylococcus aureus* could be used to demonstrate anaphylaxis to this primarily toxic fraction, since the gamma globulin would prevent the anaphylactoid reaction but allow anaphylaxis to occur. This has been demonstrated in the present experiments. Apparently the fraction A of the hemolytic *Staphylococcus aureus* strain (F-10) is less antigenic than its corresponding fraction A derived from the *Staphylococcus albus* strain (F-7). Anaphylaxis occurred sooner and persisted longer in the animals sensitized with A (F-7) than in those sensitized with A (F-10). (Almost identical dosages were used throughout the experiments.)

It is evident from the results of the cross-anaphylaxis studies that there is a close immunologic relationship between the corresponding nucleoprotein fractions from these two contrasting strains of staphylococcus. The staphylococcus

strain F-10 is a hemolytic *Staphylococcus aureus* which is coagulase positive, mannitol-fermenting, and pathogenic for rabbits. The F-7 strain is a *Staphylococcus albus;* it is coagulase negative, it does not ferment mannitol, and it is nonpathogenic for rabbits. In addition, the nucleoprotein fractions A from these two strains differ strikingly from each other with regard to their effect upon the isolated ileum of normal guinea pigs, F-10 producing an anaphylactoid effect and F-7 being totally without effect.[5]

CONCLUSION

Anaphylaxis to a primarily toxic nucleoprotein fraction derived from washed, ground, hemolytic *Staphylococcus aureus* (F-10) organisms has been demonstrated in the isolated segment of guinea pig ileum. This was done by challenging the ileum segment with nucleoprotein fraction in the presence of pooled human gamma globulin, which protects the ileum against the primary toxic action but has no such suppressive effect upon anaphylaxis.

Anaphylaxis was also demonstrated with a similarly obtained nontoxic nucleoprotein fraction derived from a strain (F-7) of *Staphylococcus albus.*

The nucleoprotein fraction from the albus strain was more allergenic than that from the aureus strain. Anaphylaxis was produced in three times as many animals; it occurred sooner and persisted longer in the albus (F-7) group than in the aureus (F-10) group.

Segments of ileum from guinea pigs sensitized to either fraction reacted anaphylactically to the other. Furthermore, cross anaphylaxis and desensitization with the heterologous fraction resulted in complete or partial desensitization to the homologous fraction. Therefore, it appears that these fractions, although derived from dissimilar strains of staphylococcus, are immunologically closely related.

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50 EAST 78TH ST.

A METHOD FOR MEASURING NASAL RESISTANCE WITHOUT INTRANASAL INSTRUMENTATION

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INTRODUCTION

PATHOLOGIC and physiologic changes in resistance of the nasal airway are difficult to measure with instruments placed in the nares, as the apparatus used for measurement distorts the orifice, creates resistance to flow, and may stimulate mucosal congestion. The problem is worse in the evaluation of vasomotor rhinitis, for a low threshold to physical stimuli is characteristic of this condition.

The first measurments of nasal resistance without intranasal instrumentation were made either in the nasopharynx or in front of the nares. Spiess,[1] in 1900, made pressure measurements in the nasopharynx with a cannula during normal respiration. In 1939 Scheideler[2] placed a double-lumen tube in the nasopharynx and, with the subject holding his breath, he measured pressure with the inner tube while a constant suction in the outer tube pulled air through the nasal passages.

Zwaardemaker,[3] in 1889, placed a cool mirror beneath the nares and estimated the respective patency of the two sides by the area and persistence of the condensed moisture on the mirror. This device is better known as Glatzel's (1901) plate because of the latter's modification and utilization of the method.

Uddströmer,[4] in 1940, divided a face mask into an oral and a nasal compartment which, in turn, were connected to gas meters. These measured the total air passing through the mouth and the nose during rest and exercise.

In order to study the response of the turbinates to physical and allergenic stimuli, we developed a posterior rhinomanometric method for measuring nasal resistance. We reversed Scheideler's suction method, which often requires local anesthesia and careful positioning of the cannula in the nasopharynx to prevent occlusion by the uvula, and introduced a flow of gas into the pharynx. In this way the cannula does not become obstructed as easily and, therefore, can be introduced more anteriorly in the pharynx where it causes no discomfort or gag reflex. With this method the apparatus is placed in the mouth and oropharynx, leaving the nose entirely free of instruments and thus accessible for stimulation with allergens and physical agents.

The method depends upon the fact that resistance to flow of gas through a tubular structure is manifest as the pressure difference between gas at the entry

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and at the exit ports of that structure. Resistance to gas flow varies with flow rate, composition of the gas, and length and diameter of the structure. Therefore, if a gas of constant composition is delivered at a constant rate of flow through a structure with unchanging length, changes in pressure resistance will reflect a change in diameter of the structure.

MATERIALS AND METHODS

We insert two tubes, 1/4 inch in diameter, through a mouthpiece into the oropharynx of the test subject. One tube delivers humidified oxygen at a constant flow rate, usually 10 liters per minute. The oxygen passes through a Venturi type of humidifier and a rotameter. The second tube, which is made of tygon, transmits pressure from the oropharynx to a differential pressure manometer which uses a Schaevitz linear differential transformer as a sensing element. The other orifice of the manometer is open to ambient pressure. The output from the manometer is directed through a strain gauge amplifier and recorded on a direct-writing oscillographic recorder.

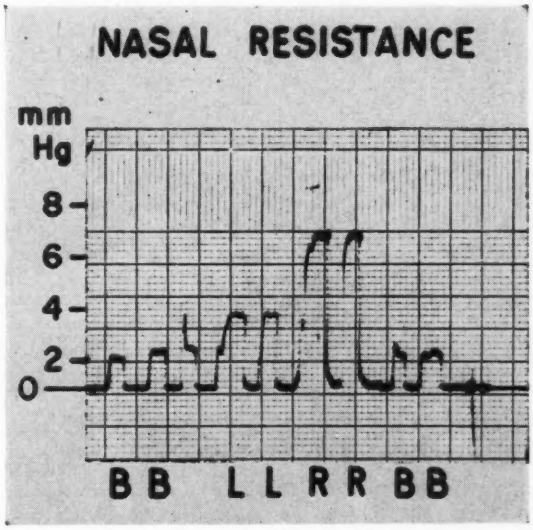

Fig. 1.—Tracing of duplicate pressure measurements from the oropharynx, recorded at the end of expiration during the flow of humidified oxygen at 10 liters per minute through the nose. *B*, Both nares open; *L*, right naris closed; *R*, left naris closed.

We instruct the subject to keep his cheeks from puffing and the lips tightly closed around the mouthpiece. The oxygen is introduced only during a voluntary inspiratory or expiratory pause while the chest is immobile. This insures the flow of all the oxygen through the nose to the outside. When both nares are open, the resistance of the entire nose is observed. The resistance of each individual side of the nose is measured by gentle obstruction with a small rubber tampon against the contralateral naris.

The apparatus was calibrated against a water manometer and the results were converted to millimeters of mercury.

The apparatus was used on subjects before and after provoking congestion with trauma and again after spraying 1 per cent ephedrine sulfate into the obstructed nasal passage.

DISCUSSION

Fig. 1 shows a representative recording. This subject had a wide difference in resistance in the two sides of his nose.

When the turbinates were congested, the pressure increased; when they were decongested with a vasoconstrictor, it decreased. The effects of vasoconstriction are shown in Fig. 2.

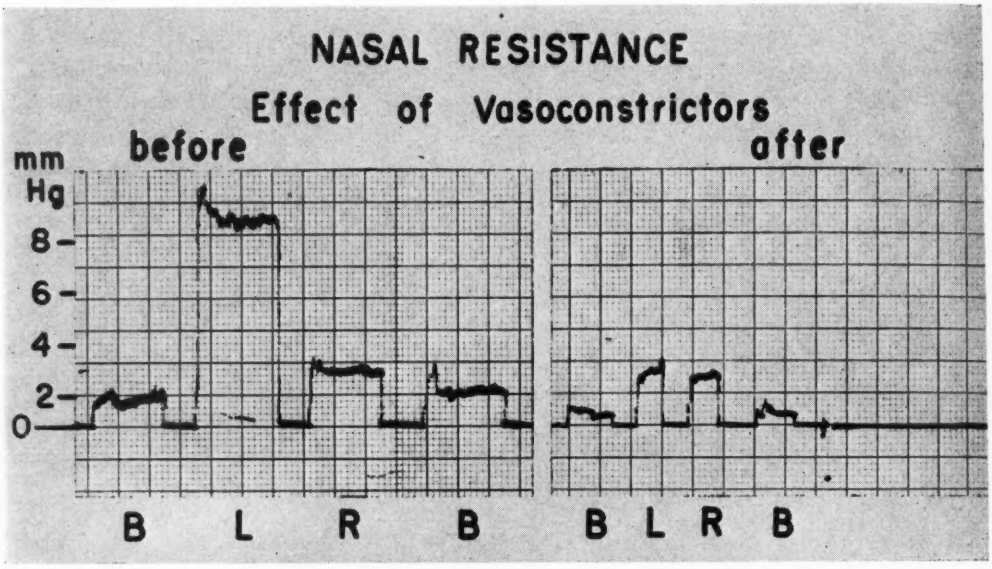

Fig. 2.—Tracing of pressure measurements from the oropharynx, recorded at the end of expiration during the flow of humidified oxygen at 10 liters per minute through the nose. The left naris was narrowed by turbinate congestion. Five minutes after application of 1 per cent ephedrine sulfate to the turbinates, the pressure in the oropharynx fell to the same level as that found in the right nasal passage.

The test subjects learned to control the apneic periods without difficulty. They reported no perception of the air flow in the nose, although such a sensation of flow was present in the pharynx. The short duration of the flow did not produce subjective drying in the pharynx or nose, and there was no measurable change in turbinate size resulting from the air flow alone.

SUMMARY

A method of measuring nasal resistance, without intranasal instrumentation, that neither disturbs turbinate size nor obstructs air flow is described. Its usefulness in the study of physiologic responses to physical and allergenic stimuli in vasomotor rhinitis is suggested.

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AN EVALUATION OF ATMOSPHERIC MOLD SPORES AS ALLERGENS IN THE NEW YORK METROPOLITAN AREA: A SEVEN-YEAR STUDY

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IN THIS country studies in mold allergy date back to 1930, when Hopkins and co-workers[1] described asthma caused by sensitivity to alternaria. Following this, Brown,[2] Cobe,[3] Prince[4] and his associates, and others[5] reported on mold sensitivity as a causative factor in hay fever and asthma.

In 1936, Feinberg and his associates,[6] working in the Chicago area, stated that the problem of mold allergy was very much like the problem of pollen allergy. They found alternaria to be the most important mold offender and were able to correlate clinical symptoms with mold counts. At about the same time Peshkin,[7] working in New York, reported that he was unable to draw any definite conclusions as to the allergenic significance of molds in this area on the basis of his study covering over 1,200 cases. Bernton and Thom,[7] on the other hand, considered these allergens important enough to warrant the performance of mold tests as a routine procedure in inhalant allergies. VanderVeer[8] recognized the clinical importance of molds but could not correlate symptoms with the mold counts reported for the New York area by Durham[9] from 1933 to 1936.

In 1942, a questionnaire on the importance of mold allergy was sent to all members of the Society for the Study of Asthma and Allied Condition.[10] In this survey only a few allergists in the New York metropolitan area considered molds of definite clinical significance.

Prior to 1948, the lack of a standard technique for counting atmospheric mold spores made it difficult to study the clinical importance of molds as allergens. When, in 1948, the Research Council of The American Academy of Allergy adopted the same technique for daily mold spore counts which it had previously recommended for pollen counts, one of us (D. M.[11]) started making daily mold spore counts at the Allergy Laboratory of The Jewish Hospital of Brooklyn. The counts were done from April to November, and in some years perennially. Slides were exposed in Durham pollen slide shelters at ten different stations in the New York metropolitan area during from one to seven mold seasons. On the basis of these daily counts, it was possible to correlate clearly the patients' symptoms with the mold content of the air. In this way the importance of mold sensitivity as a cause of seasonal allergic rhinitis and bronchial asthma in the New York area was definitely established. This

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was the first time that the gravity slide method of spore counting was used for making a protracted mold survey of a large metropolitan area. In prior surveys data had been obtained primarily from the study of culture plates. We have worked with both methods and are convinced that the slide method is the more useful in the clinical study of mold-sensitive cases. The reasons for our choice of methods will be discussed presently.

On the basis of seven years of these mold counts, a characteristic pattern for the mold season in the New York metropolitan area was recognized. The spores of alternaria and hormodendrum proved to be the ones most frequently found on the exposed slides. The averages of the seasonal curves for alternaria and hormodendrum in Brooklyn, during the seven-year period from 1948 to 1954 inclusive, are presented in Fig. 1. The chronological relationship of the

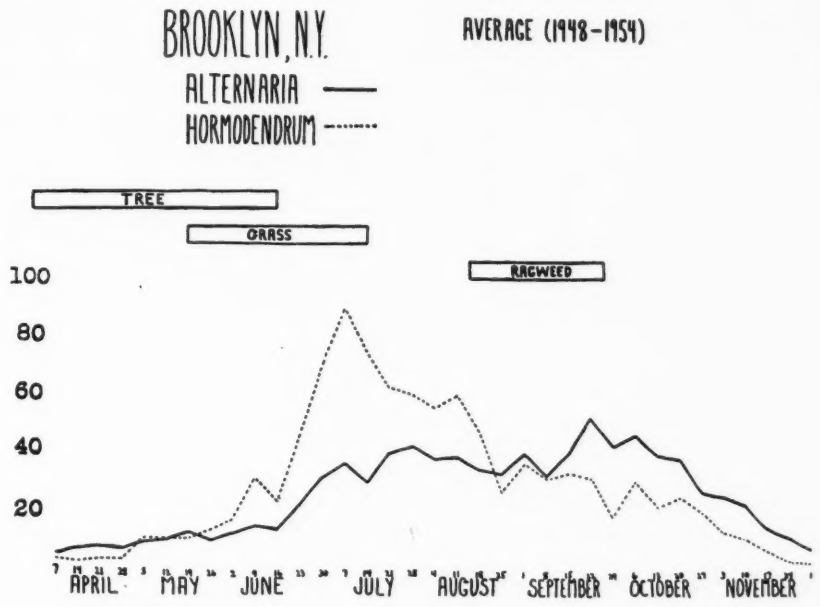

Fig. 1.

mold seasons to the pollinating periods for trees, grasses, and ragweed is also illustrated on this chart. The counts are expressed in terms of number of spores per square centimeter of exposed slide. Fig. 2 presents the averages of the totals for each month for the two types of spores at the Brooklyn station from April to November, inclusive, during the seven-year period. Fig. 3 illustrates the variations in the monthly totals for alternaria at seven different stations in the New York metropolitan area during a single mold season (1954). In this seven-year study, it was found that alternaria spore counts were higher on the south shore (Rockaway and Bay Shore) and north shore (Huntington and Northport) of Long Island and in the northeastern section of New Jersey (New Brunswick) than in Brooklyn and Manhattan (Battery). However, in Bridgeport and Stamford, in Connecticut, the counts were lower than those in Brooklyn and Manhattan.

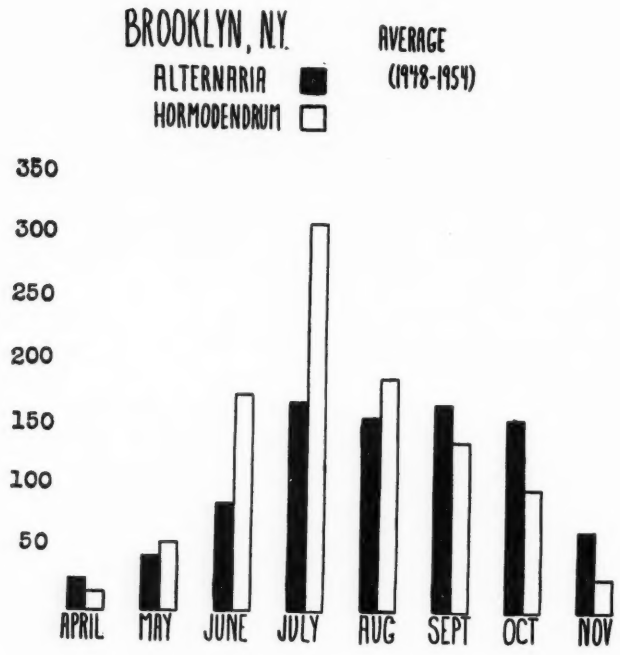
BROOKLYN, N.Y.
AVERAGE
(1948-1954)
ALTERNARIA
HORMODENDRUM
350
300
250
200
150
100
50
APRIL
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV

Fig. 2.

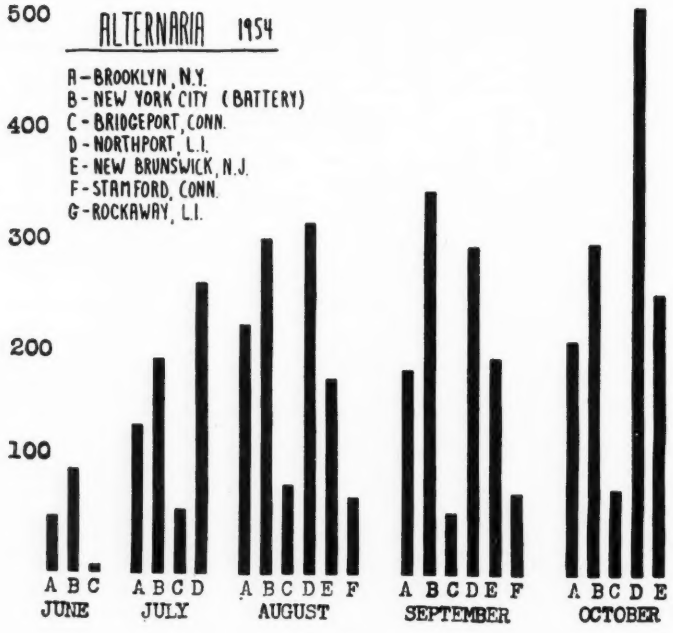
ALTERNARIA 1954
A - BROOKLYN, N.Y.
B - NEW YORK CITY (BATTERY)
C - BRIDGEPORT, CONN.
D - NORTHPORT, L.I.
E - NEW BRUNSWICK, N.J.
F - STAMFORD, CONN.
G - ROCKAWAY, L.I.
500
400
300
200
100
A B C
JUNE
A B C D
JULY
A B C D E F
AUGUST
A B C D E F
SEPTEMBER
A B C D E
OCTOBER

Fig. 3.

Studies were made on various clinical aspects of the mold allergy problem in the New York area. Fifty-five patients in the Allergy Clinic of The Jewish Hospital of Brooklyn, in whom clinical mold sensitivity was definitely established, were analyzed on the basis of age distribution, symptomatology, seasonal incidence, skin reactions to molds, skin reactions to pollens, passive transfer tests, conjunctival reactions, dosage tolerance, and other considerations. In each case a complete medical work-up was done while the patient was undergoing these allergy studies. The period of observation for each patient was from one to three mold seasons.

Intracutaneous tests were done with the following mold extracts: alternaria, hormodendrum, aspergillus, penicillium, helminthosporium, yeast, and mushroom. Only the yeast and mushroom extracts were prepared in the Allergy Laboratory of The Jewish Hospital of Brooklyn; all others were obtained from Abbott Laboratories. Tests were done with a 1:1,000 dilution of the mold extract and, when these were negative or weakly positive, with a 1:100 dilution. Buffered saline was used in making the dilutions of the mold extracts. Positive reactions were read as slight, moderate, or marked, according to the usual standards for intracutaneous testing. The glycero-saline solution used by Abbott Laboratories in the preparation of their extracts was used as a control for the intracutaneous and ophthalmic tests. Dilutions of glycero-saline solution were made with buffered saline.

In eleven patients, most of whom showed multiple positive reactions to various mold extracts, passive transfer tests were done by the technique described by Walzer.[12]

Conjunctival tests with mold extracts were done in almost all cases. One eye was used for mold tests and the other for the controls. When the instillation into the eye of one drop of a 1:1,000 dilution gave a negative reaction, the 1:100 dilution was tried. The usual precautions associated with conjunctival testing were observed. Reactions were read as 1+, 2+, or 3+, depending upon the amount of congestion and edema of the conjunctiva produced by the allergen test. Every effort was made to avoid excessive ophthalmic reactions. The control tests were made with the same materials that were used for controls in skin testing.

ANALYSIS OF CASES

Age, Sex, and Race.—The fifty-five mold-sensitive patients studied ranged in age from 3 to 49 years. They included thirty-four male and twenty-one female patients, of whom forty-six were white and nine were Negroes. Seventeen years. Forty-three of the fifty-five patients studied (78 per cent) were under patients had had symptoms for two to five years, and ten for more than five 10 years of age. There were nine patients (17 per cent) between 11 and 20 years, and only three (5 per cent) were over 20 years of age.

Nature of Symptoms.—In all cases in this series the respiratory tract was involved in the symptomatology (Table I). Ten (18 per cent) of the patients

complained only of asthma, twenty-one (38 per cent) had only nasal symptoms, and twenty-four (44 per cent) had both. In all, 62 per cent of the patients had asthmatic symptoms and 82 per cent had allergic rhinitis. Involvement of other organs or systems of the body was infrequent and mild.

TABLE I. TYPES OF SYMPTOMS IN FIFTY-FIVE CASES OF MOLD ALLERGY

TYPE OF SYMPTOMS	NO. OF CASES	PER CENT
Nasal and bronchial (asthma) symptoms	24	44
Nasal symptoms only	21	38
Bronchial symptoms only	10	18
Total number of cases with nasal symptoms	45	82
Total number of cases with bronchial symptoms	34	62

Correlation of Mold and Pollen Sensitivities.—In this series sixteen (29 per cent) of the patients who were sensitive to molds had no pollen sensitivity. Of the thirty-nine (71 per cent) who had pollen reactions (Table II), twenty-one (38 per cent) were sensitive to ragweed only and sixteen (29 per cent) were sensitive to both ragweed and timothy. Only 2 (4 per cent) were sensitive to timothy alone. One patient, sensitive to ragweed and timothy, was also tree-sensitive.

TABLE II. ASSOCIATED POLLEN SENSITIVITIES IN FIFTY-FIVE PATIENTS WITH MOLD ALLERGY

	NO. OF CASES	PER CENT
Patients without pollen sensitivity	16	29
Patients with pollen sensitivity	39	71
Patients sensitive only to ragweed	21	38
Patients sensitive only to timothy	2	4
Patients sensitive to timothy and ragweed	16	29
Total number of patients sensitive to ragweed	37	67
Total number of patients sensitive to timothy	18	33

Results of Intracutaneous Tests With Molds.—In this series eigthteen patients showed a marked reaction to a dilution of 1:1,000 of alternaria and twenty-three had moderate reactions, making a total of 75 per cent of patients with strongly positive reactions to this dilution (Table III). There were fourteen slight reactions and no negative reactions to the 1:1,000 dilution. Fifty-two patients were tested with the 1:100 dilution of alternaria extract. Of these, forty-nine showed marked reactions and three showed moderate reactions. Thus, all patients in this series gave strong reactions to the 1:100 test solution of alternaria. Alternaria, therefore, was the outstanding offender among the mold-sensitive patients in the New York area.

Testing with hormodendrum revealed one marked reaction and three moderate reactions with the 1:1,000 dilution, making a total of 7 per cent with strongly positive reactions. There were twenty-four negative reactions and twenty-seven slight reactions. Fifty-four patients were tested with the 1:100 dilution of hormodendrum and seven marked and ten moderate reactions were obtained, making a total of 31 per cent with strongly positive reactions. There

TABLE III. RESULTS OF INTRACUTANEOUS TESTS WITH ALTERNARIA AND HORMODENDRUM

MOLD	DILUTION	NO. TESTED	SIZE OF REACTION			NEGATIVE	PER CENT STRONGLY POSITIVE, MARKED OR MODERATE
			MARKED	MODERATE	SLIGHT		
Alternaria	1:1,000	55	18	23	14	0	75
Alternaria	1:100	52	49	3	0	0	100
Hormodendrum	1:1,000	55	1	3	27	24	7
Hormodendrum	1:100	54	7	10	27	10	31
Glycero-saline solution	1:1,000	55	0	0	1	54	0
Glycero-saline solution	1:100	54	0	0	10	44	0

were twenty-seven slight reactions and ten negative reactions with the 1:100 dilution. Hence, in this series positive reactions to hormodendrum were neither as prevalent nor as pronounced as those to alternaria.

With the control tests made with the glycero-saline diluent, one slight reaction with the 1:1,000 dilution and ten slight reactions with the 1:100 dilution were obtained. In every case in which the control was positive, the final reading for the mold test was computed by subtracting the amount of the control reaction from that of the mold reaction.

TABLE IV. RESULTS OF INTRACUTANEOUS TESTS WITH MISCELLANEOUS MOLDS

MOLD	DILUTION	NO. TESTED	SIZE OF REACTION			NEGATIVE	PER CENT STRONGLY POSITIVE, MARKED OR MODERATE
			MARKED	MODERATE	SLIGHT		
Aspergillus	1:1,000	47	1	2	15	29	6
Aspergillus	1:100	48	3	6	25	14	19
Penicillium	1:1,000	51	0	1	26	24	2
Penicillium	1:100	47	4	4	34	5	17
Helmintho-sporium	1:1,000	19	0	6	10	3	32
Helmintho-sporium	1:100	17	5	6	5	1	65
Yeast	1:50	50	1	4	28	17	10
Yeast	1:10	48	8	8	24	8	33
Mushroom	1:10	48	0	0	12	36	0

The results obtained with intracutaneous tests with other molds are listed in Table IV. It is of interest to note that tests with a 1:100 dilution of helminthosporium produced strongly positive reactions in 65 per cent of nineteen cases tested. Strongly positive reactions to aspergillus in a 1:100 dilution were obtained in only 19 per cent of the forty-eight cases tested. Penicillium (1:100) produced 17 per cent of strongly positive reactions and yeast (1:10) 33 per cent. Mushroom (1:10) elicited no strong positive reactions in forty-eight cases tested.

Passive Transfer Tests.—Passive transfer tests were done on eleven patients, most of whom showed multiple strong positive skin reactions to molds in tests

with a 1:100 dilution (Table V). Transfers were successful in all tests with alternaria (eleven cases), hormodendrum (six cases), aspergillus (three cases), and helminthosporium (two cases). Penicillium transfers were successful in three out of four cases (75 per cent) and yeast transfers in four out of five cases (80 per cent).

TABLE V. RESULTS OF PASSIVE TRANSFER TESTS IN ELEVEN CASES WITH MULTIPLE POSITIVE INTRACUTANEOUS REACTIONS TO MOLDS

MOLD TESTED	DILUTION	NO. OF CASES TESTED BY PASSIVE TRANSFER	NO. OF POSITIVE TRANSFERS	PER CENT OF POSITIVE TRANSFERS
Alternaria	1:100	11	11	100
Hormodendrum	1:100	6	6	100
Aspergillus	1:100	3	3	100
Penicillium	1:100	4	3	75
Helminthosporium	1:100	2	2	100
Yeast	1:10	5	4	80

Conjunctival Tests.—Conjunctival tests were done on forty-seven of the fifty-five patients studied (Table VI). With alternaria 11 per cent of the patients showed positive reactions with the 1:1,000 dilution and 51 per cent with the 1:100 dilution, making a total of 62 per cent positive. With hormodendrum only 3 per cent were positive with the 1:1,000 dilution and 18 per cent were positive with the 1:100 dilution, making a total of 21 per cent positive. Control tests in the forty-seven cases were negative. Most of the conjunctival reactions obtained were weak positives (+ or ±).

TABLE VI. RESULTS OF CONJUNCTIVAL TESTS ON FORTY-SEVEN PATIENTS WITH MOLD SENSITIVITY

MOLD	NO. OF CASES TESTED	POSITIVE REACTIONS						NEGATIVE REACTIONS	
		DILUTION OF 1:1,000		DILUTION OF 1:100		TOTAL			
		NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT
Alternaria	47	5	11	24	51	29	62	18	38
Hormodendrum	38	1	3	7	18	8	21	30	79
Aspergillus	2			1	50	1	50	1	50
Penicillium	2							2	100
Yeast 1:10	5					3	60	2	40
Controls	47							47	100

There were thirteen strongly positive reactions to alternaria, two of which were obtained with the 1:1,000 dilution. There were three strongly positive reactions to hormodendrum, one of which was obtained with the 1:1,000 dilution. The strongest eye reactions were obtained with alternaria extract. In the patients who were clinically sensitive to both molds and pollens, there was a much higher incidence of positive conjunctival reactions to alternaria than to ragweed or timothy, even when high concentrations of these pollens were used.

Constitutional Reactions to Treatment.—Thirty-five patients who were clinically sensitive to alternaria were given specific therapy. Eight patients developed constitutional reactions at some time during their course of treatment, some at very low dosage levels (Table VII). Six of these eight patients showed

definite (one plus or stronger) conjunctival reactions, whereas in the twenty-seven patients who experienced no constitutional reactions there were only three cases of positive conjunctival reaction of equivalent intensity. This suggests that there is a higher degree of conjunctival or mucosal reactivity in patients who are subject to constitutional reactions than in those who take their dosage well. A similar finding was reported by one of us (H. S.[13]) in relation to constitutional reactions following pollen injections.

TABLE VII. THE RELATIONSHIP OF CONJUNCTIVAL AND CONSTITUTIONAL REACTIONS IN PATIENTS TREATED WITH ALTERNARIA

CASES SHOWING CONSTITUTIONAL REACTIONS			CASES WITHOUT CONSTITUTIONAL REACTIONS		
	RESULTS OF CONJUNCTIVAL TESTS WITH:			RESULTS OF CONJUNCTIVAL TESTS WITH:	
CASE	DILUTION OF 1:1,000	DILUTION OF 1:100	CASE	DILUTION OF 1:1,000	DILUTION OF 1:100
1	0 ±	+	1	0 ±	+ +
2	+ ±	+ +	2	0	+
3	0	0	3	0	+
4	0	±	4	0	±
5	0	+	5	0	0 ±
6		+	6	0	0 ±
7		+	7	0	0 ±
8		+ + +	8		0 ±
			9		0
			10 to 27		0

In eight (23 per cent) of the thirty-five patients treated, constitutional reactions occurred following injections with alternaria extract. All of these patients were children. This incidence of constitutional reactions to molds is far in excess of that seen among children treated with ragweed, in whom constitutional reactions tend to occur infrequently.

Comparison of Gravity Slide and Plate Methods of Mold Spore Counting.— The mold spore counts in these studies were obtained from examinations of slides exposed daily from April through November. This method of counting presents certain advantages over the culture plate method. It probably yields the more useful information, as the slides are exposed continuously throughout the season. Plates, on the other hand, are exposed for periods of only twelve minutes each day.

Other advantages of the slide method include the following: (1) it facilitates the correlation of patients' symptoms with the daily fluctuations in the spore counts; (2) pollen granules and mold spores may be counted simultaneously on the same slide, thus affording a daily correlation of pollen and mold concentrations of the air; (3) slides may be stored for convenience without fear of changes in the deposited particles on the slide, thus making it feasible to mail exposed slides from various stations in the surveyed area to the laboratory for counting; (4) the alternaria spore, which is generally recognized to be the most important atmospheric mold from the clinical standpoint, is readily identifiable on the slides. The spores of hormodendrum, helminthosporium, acrotheceium, smuts, and rusts may also be readily identified.

A disadvantage of the slide method is the fact that the material on the slide may be washed away by rain or blown away by strong winds. Another disadvantage is its failure to yield information concerning some molds. The spores of aspergillus, penicillium, mucor, rhizopus, fusarium, and yeasts are identifiable only from colonies on culture plates. On the other hand, the plates must be examined within three to five days after exposure, or they may become overgrown with colonies and identification may become difficult or impossible. Furthermore, the short period of plate exposure (twelve minutes), may not yield a representative sample of atmospheric spore conditions as they occur during the entire twenty-four hours.

It is thus apparent that neither method is completely adequate in itself. However, the two methods may be used to complement each other. To obtain as complete a picture as possible of the atmospheric mold spore conditions in a given area, both methods might be used simultaneously to great advantage.[14]

Correlation of Mold Counts and Clinical Symptoms.—

Alternaria: We could definitely correlate the patients' seasonal symptoms with the variations in alternaria spore counts in almost all cases. On days when the counts were high there was an exacerbation of symptoms in most of the patients, and there was a diminution or absence of symptoms on days when the counts were low.

Hormodendrum: A correlation between hormodendrum counts and clinical symptoms, similar to that seen with alternaria, was not apparent in our series of patients. On days when hormodendrum counts were high and alternaria counts were low, there was no increase in symptoms. In fact, there were no patients in this series who proved to be sensitive to hormodendrum alone. One of us (D. M.) has seen only one patient, not a member of this series, in whom hormodendrum sensitivity occurred without any other mold sensitivity and in whom clinical sensitivity could be correlated with the counts. This patient (R. G.), a boy 12 years of age, had a history of spring and fall hay fever and asthma of six years' duration, with positive intracutaneous reactions to timothy and ragweed. During the second year of therapy, it was noted that he had nasal and bronchial symptoms during late June and July, when the daily slide counts showed high concentrations of hormodendrum spores and an absence of all pollen granules. Intracutaneous tests revealed strongly positive reactions to hormodendrum and negative reactions to alternaria. Repeated testing with 1:10,000, 1:1,000 and 1:100 dilutions of these mold extracts confirmed the original findings.

DISCUSSION

The mold spore surveys done with the standard technique in the New York metropolitan area during the past seven years have demonstrated the existence of a definite mold season in this district beginning about the last week of June and ending about the second week of November. During the tree and grass

pollinating periods there are only small amounts of alternaria and hormodendrum spores in the atmosphere. Increased numbers of spores begin to appear at the end of the grass pollinating season (late in June) and continue in high concentrations throughout the ragweed season, outlasting this pollen by six or more weeks (Fig. 1).

It is evident that there is need for careful study and differential diagnosis in the management of this type of patient. Many of the mold patients in this series had previously been treated either as pollen cases or as seasonal food-sensitivity cases and had fared poorly. The same may be said of many of the patients with combined mold and pollen sensitivity who had previously been treated for pollen sensitivity alone.

In our studies we were impressed with the high incidence of mold sensitivity in the younger age group. Seventy-eight per cent of our patients were under 10 years of age. Feinberg, in his studies, found a similar age distribution.

The incidence of bronchial asthma as a symptom in 62 per cent of our cases and as the sole symptom in 18 per cent is another interesting finding. This is a higher incidence of asthma than is usually found in pollinosis and is in accord with the reports of other investigators.

The results of skin testing with alternaria in our series were similar to those obtained by Feinberg in Chicago in a much larger series of cases. In our series we found no instance of a marked positive reaction to hormodendrum or to any other of the molds tested, in the absence of a marked reaction to alternaria. Only three of our patients revealed strongly positive reactions to all of the six molds tested.

Strongly positive intracutaneous and ophthalmic reactions to hormodendrum were about one-third as frequent as those to alternaria. In six cases studied, positive transfers were obtained with hormodendrum in all instances. In the light of this evidence of active antigenicity, it is difficult to understand why clinically proved hormodendrum cases are rare in this area.

We are of the opinion that the limitations of the counting technique used in this study may explain part of the discrepancy. Hormodendrum spores are seen on the slides in clumps rather than as separate spores, especially during late June and almost all of July. Small clumps may contain five or six spores and large ones as many as forty to eighty or more spores. Therefore, the figures obtained by counting individual spores in such clumps may not have the same significance as those obtained by counting spores that fall on the slide singly, such as those of alternaria. If total daily counts were figured in terms of numbers of clumps rather than as numbers of individual spores, our counts for hormodendrum would be much lower than those shown in Figs. 1 and 2.

The high incidence of positive reactions obtained with helminthosporium (65 per cent in nineteen cases tested) may be explained on the basis of a possible antigenic relationship between this mold and alternaria.[14]

Only one of our patients had pronounced eye symptoms during the mold season. This absence of ophthalmic symptoms in mold-sensitive patients has

been noted previously by Tuft and Blumstein.[15] This lack of eye symptoms in mold-sensitive patients who show positive conjunctival reactions to ophthalmic tests is not readily explainable. In six patients with negative eye tests to alternaria, strong positive skin reactions were elicited which could be confirmed by passive transfer tests. Hence, the eye test does not appear to be a reliable index of clinical sensitivity or of the need for therapy in mold sensitivity.[16]

We are impressed with the importance of household environmental molds, such as aspergillus, penicillium, etc., as causes of clinical sensitivity and with the role of house dust as a factor in mold sensitivity. This subject, which has been discussed by Christensen[17] and other investigators,[18] is under investigation and will be presented in a future communication.

SUMMARY

Fifty-five mold-sensitive patients, residing in the New York metropolitan area, and ranging in age from 3 to 49 years, were studied. This condition was found most frequently in the younger age group, 78 per cent occurring in the first decade of life and 17 per cent in the second decade. Bronchial asthma was found in 62 per cent of the cases. It appeared as the sole symptom in 18 per cent, a higher incidence than occurs in pollinosis. Conjunctival symptoms were noticeably absent. Mold sensitivity occurred alone in 29 per cent and was associated with pollen sensitivity (usually ragweed) in 71 per cent of the cases. All of the patients in this series showed strongly positive reactions to alternaria. Positive reactions were also obtained to hormodendrum, yeast, aspergillus, penicillium, and helminthosporium. Positive reactions to the molds were confirmed by passive transfer in almost 100 per cent of the cases. Conjunctival tests were positive in 62 per cent of the patients tested with alternaria and in 21 per cent tested with hormodendrum. Positive conjunctival tests were also obtained to yeast and aspergillus, but not to mushroom. Constitutional reactions to treatment doses occurred frequently, usually in children.

CONCLUSIONS

1. Mold sensitivity is an important cause of respiratory allergy in the New York metropolitan area from about the middle of June to about mid-November.

2. Alternaria and hormodendrum spores are the ones most commonly found in slide counts in this area. Of these, alternaria is by far the more important from the antigenic, immunologic, and clinical points of view. Clinical sensitivity to hormodendrum alone is rarely seen in this area.

3. In our studies, daily slide counts of the mold content of the air were found to be more informative than other methods in the diagnosis and treatment of mold sensitivity.

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COMPARATIVE EFFECTS OF AEROSOL BRONCHODILATORS ON VENTILATORY FUNCTION IN BRONCHIAL ASTHMA AND CHRONIC PULMONARY EMPHYSEMA

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THE inhalation of nebulized bronchodilators is a rapid method of administration of spasmolytic drugs for the treatment of asthma and emphysema. The effectiveness of aerosol therapy has been attributed to the deposition of medication at the site of desired action in the lungs and tracheobronchial tree. During the past year, isoproterenol hydrochloride has become available for administration in a new form of self-propelling aerosol (Medihaler-Iso) and has been reported by Freedman[1] as an effective bronchodilator in a preliminary clinical trial in forty-two cases of bronchial asthma. To determine whether or not this form of isoproterenol hydrochloride is a bronchodilator, and to compare it with racemic epinephrine hydrochloride (Vaponefrin), a known bronchodilator,[2] a group of twenty-six patients with chronic bronchopulmonary disease was studied.

The distribution of aerosols to the bronchi is influenced by numerous factors, including (1) those dependent upon the nature of the aerosol, such as the particle size, temperature, and chemical characteristics of the mist, and the type of nebulization, and (2) those dependent upon the patient, such as the amount of bronchospasm present, the presence or absence of secretions within the bronchi, and the severity of his disease.[1] Because of the widening of the tracheobronchial tree produced by positive pressure breathing, aerosols have been administered via this route in the hope of achieving more effective distribution of the drug. Segal and associates[3] compared the average increase in vital capacity attained in 132 patients after inhalations of the bronchodilator, Dapanone, to that of thirty-two patients treated with intermittent positive pressure breathing (IPPB) and Dapanone, and concluded that the combination of IPPB with a bronchodilator spray was more effective than the bronchodilator spray alone. Wu and colleagues[4] report a similar advantage to bronchodilator administered with IPPB compared to bronchodilator via oxygen mask. However, Miller and associates[5] noted no consistent difference when Isuprel aerosol was administered with or without IPPB, and tabulated similar results in 600 patients studied by other investigators. The studies by Leslie and co-workers[6] of thirty-three patients with moderate to advanced pulmonary emphysema

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confirm the findings of Miller and associates that: "The addition of intermittent positive pressure breathing did not enhance the value of bronchodilator therapy. No difference in average improvement after bronchodilator therapy could be demonstrated whether or not intermittent positive pressure breathing was used."

The present study was designed not only to evaluate the effectiveness of isoproterenol as a bronchodilator, particularly as compared to epinephrine, but also to assess the additional effect, if any, of IPPB.

MATERIALS AND METHODS

The eleven asthmatic and fifteen patients with emphysema included in this study were selected on the basis of a clinical diagnosis and pulmonary function studies which corroborated the impression of asthma or emphysema. Progressive exertional dyspnea, cough, and expectoration were prominent features in the history of the emphysema patients, and widened AP diameter of the chest, limited thoracic motion, and distant breath sounds were evident on physical examination. Ventilatory studies, which revealed a decreased vital capacity and marked expiratory delay, corroborated the diagnosis of emphysema. The asthmatic patients gave a history of attacks of wheezing and acute dyspnea distinct from paroxysmal nocturnal dyspnea. These attacks were precipitated by known allergens or respiratory infections, and were symptomatically improved by bronchodilator therapy. Physical examination during the symptom-free interval usually revealed prolonged expiration and wheezes during forced breathing. Pulmonary function studies were more variable than in the emphysema patients, but usually demonstrated some expiratory delay. All patients included in the emphysema group were males and all were smokers. Among the asthmatic patients there were two females and one nonsmoker. Although nine patients took maintenance digitalis therapy for congestive heart failure secondary to cor pulmonale, there was no dyspnea attributable to cardiac decompensation in the patients in this study.

Three measurements of pulmonary function were made: vital capacity, one-second vital capacity,[7] and mean midexpiratory flow rate (MMF).[8] The latter was preferred to the maximum breathing capacity as a means of evaluating airway obstruction, as it is reproducible and causes no distress to the patient.

The medications used were epinephrine in the form of Vaponefrin, which is a 2.25 per cent solution of racemic epinephrine hydrochloride with ½ to 1 per cent chlorbutanol as a preservative, isoproterenol in the form of Medihaler-Iso,* which is a 0.25 per cent solution of isoproterenol hydrochloride in alcohol with an inert propellant, and isoproterenol-placebo, containing merely the propellant and the vehicle without isoproterenol. The particle size is reported as 2.5 micra for epinephrine and less than 3 micra for isoproterenol,[9] both within the optimum range for nebulization into the smaller bronchi.[10] Epinephrine, 0.5 c.c. diluted to 1 c.c. with water, was vaporized by means of a Vaponefrin hand nebulizer or an Emerson IPPB valve at 10 to 20 cm. of water.

*Medihaler used in this study was supplied by courtesy of Riker Laboratories, Inc., Los Angeles, California.

Isoproterenol was administered through a plastic adapter and valve which dispenses a uniform dose of 0.06 mg. A similar volume of placebo was administered through the same type of adapter and valve. The patients received no bronchodilator aerosols for at least three, and usually six, hours before the test. Only one medication was tested in any one day. At two- to four-week intervals, spirometry was performed in accord with one of the following programs:

1. Two measurements before and two after the administration of placebo, using four inhalations with at least a one- to two-minute waiting period before repeat testing after administration of the medication.
2. Two measurements before and two after isoproterenol, using four inhalations and a one- to two-minute waiting period before repeat testing.
3. Two measurements before and after the administration of epinephrine with a Vaponefrin hand nebulizer, at least four inhalations (eight to ten compressions of bulb of nebulizer) followed by a one- to two-minute waiting period.
4. Two measurements before and after the administration of epinephrine with IPPB, inhaled until the nebulizer was emptied (approximately ten to fifteen minutes).

Each of the four studies was carried out one to four times in each patient, and the mean results are reported. Spirograms were analyzed in terms of per cent change in vital capacity, one-second vital capacity, and MMF, and the best of two breaths before and after treatment was compared in each instance.

RESULTS

Studies in the fifteen patients with emphysema showed a lack of improvement after placebo (Table I) and, by comparison, a highly significant improvement after isoproterenol in all three tests. In patients with asthma there was slight improvement after placebo and the comparative effect of isoproterenol on MMF was probably significant, and on vital capacity and one-second vital capacity only possibly significant. Cough frequently followed the administration of aerosols and was often productive of mucous plugs or small amounts of sputum, but spirograms done after cough induced by placebo demonstrated no improvement in ventilatory function. Thus, isoproterenol has a bronchodilator effect which is independent of the dispersing vehicle, the new mode of administration, and the raising of secretions.

Table II compares the effects of isoproterenol with the effects of epinephrine nebulized manually and epinephrine with IPPB on three tests of ventilatory function in the asthma and emphysema groups. Mean per cent improvement ± standard error of the mean is presented for each group. The level of significance of the comparisons between adjacent tests is listed between them. In the patients with emphysema, there are two probably significant comparisons: (1) there was more improvement in one-second vital capacity after isoproterenol than after epinephrine nebulized manually and (2) there was more improvement

TABLE I. COMPARISON OF THE EFFECTS OF ISOPROTERENOL AND PLACEBO ON THREE TESTS OF VENTILATORY FUNCTION IN FIFTEEN PATIENTS WITH EMPHYSEMA AND ELEVEN PATIENTS WITH ASTHMA

	EMPHYSEMA			ASTHMA		
	MMF	VITAL CAPACITY	1 SECOND	MMF	VITAL CAPACITY	1 SECOND
Isoproterenol	23.5 ± 5.8	12.3 ± 2.3	17.5 ± 4.2	34.2 ± 7.1	9.5 ± 4.5	22.0 ± 7.8
Placebo	−4.5 ± 2.9	1.1 ± 1.2	−4.0 ± 1.2	1.6 ± 9.8	2.9 ± 3.4	6.9 ± 3.2
Significance level	<0.001	<0.001	<0.001	>0.001 <0.01	>0.01 <0.10	>0.01 <0.10

The mean per cent improvement ± standard error of the mean is recorded for each medication, and the level of significance of the comparison between isoproterenol and placebo is listed below each test.

in MMF after isoproterenol than after epinephrine and positive pressure. The mean per cent improvement in MMF, one-second vital capacity, and vital capacity was greater (although not significantly) after isoproterenol than after epinephrine with or without positive pressure breathing in this group. In the asthmatic patients there was no significant difference in any of the three tests with any one type of therapy as compared to the other two.

TABLE II. COMPARISON OF THE EFFECTS OF THREE FORMS OF BRONCHODILATOR THERAPY ON THREE TESTS OF VENTILATORY FUNCTION IN FIFTEEN PATIENTS WITH EMPHYSEMA AND ELEVEN PATIENTS WITH ASTHMA

	ISOPROTERENOL		EPINEPHRINE		EPINEPHRINE WITH IPPB		ISOPROTERENOL
	PER CENT IMPROVEMENT	SIGNIFICANCE LEVEL	PER CENT IMPROVEMENT	SIGNIFICANCE LEVEL	PER CENT IMPROVEMENT	SIGNIFICANCE LEVEL	PER CENT IMPROVEMENT
			Emphysema				
MMF	18.3 ± 5.4	>0.10	11.5 ± 5.5	>0.10	3.7 ± 3.3	>0.01 <0.05	18.3 ± 5.4
Vital capacity	13.7 ± 3.0	>0.10	10.3 ± 2.4	>0.10	11.6 ± 2.5	>0.10	13.7 ± 3.0
One-second volume	19.9 ± 4.6	>0.01 <0.05	7.8 ± 3.6	>0.10	16.4 ± 3.8	>0.10	19.9 ± 4.6
			Asthma				
MMF	30.6 ± 6.3	>0.10	29.8 ± 5.8	>0.10	33.0 ± 2.5	>0.10	30.6 ± 6.3
Vital capacity	7.4 ± 3.7	>0.10	8.5 ± 3.3	>0.10	8.7 ± 3.5	>0.10	7.4 ± 3.7
One-second volume	20.7 ± 6.8	>0.10	25.2 ± 6.9	>0.10	26.2 ± 7.1	>0.10	20.7 ± 6.8

Mean per cent improvement for each test ± standard error of the mean is recorded. and the level of significance for adjacent tests is listed between them. The average value of one to four tests on each patient with each form of therapy was used to calculate the mean.

Comparing the response of patients with asthma to that obtained in the emphysema group (Table III), MMF was improved by all forms of therapy in asthmatic patients (29.8 to 33.0 per cent), but to a lesser extent in the patients with emphysema (3.7 to 11.1 per cent). The insignificant difference between the improvement of MMF after isoproterenol in the asthma and emphysema groups may result from the fact that isoproterenol was the superior form of bronchodilator therapy in the emphysema group.

TABLE III. COMPARISON OF THE EFFECTS OF THREE FORMS OF BRONCHODILATOR THERAPY ON THREE TESTS OF VENTILATORY FUNCTION IN FIFTEEN PATIENTS WITH EMPHYSEMA AND ELEVEN PATIENTS WITH ASTHMA

	ISOPROTERENOL			EPINEPHRINE			EPINEPHRINE WITH IPPB		
	VITAL CAPACITY	MMF	1-SECOND	VITAL CAPACITY	MMF	1-SECOND	VITAL CAPACITY	MMF	1-SECOND
Asthma	7.4	30.6	20.7	8.5	29.8	25.2	8.7	33.0	26.2
Emphysema	13.7	18.3	19.9	10.3	11.1	7.8	11.6	3.7	16.4
Significance level	>0.10	>0.10	>0.10	>0.10	<0.05	>0.10	>0.10	<0.05	>0.10

The mean per cent improvement in each test is recorded for each group and the level of significance of the comparisons between them is recorded below.

DISCUSSION

The evaluation of bronchodilators is subject to many errors, which may be divided into those dependent upon the variability of the patients and those due to variability of the tests. In general, the emphysema patients represented a group with fairly constant day-to-day ventilatory impairment. The asthmatic patients, however, were less consistent. Lowell and his colleagues[11] observed daily variations in symptoms and pulmonary function tests (vital capacity and one-second volume) of five asthmatic patients at weekly intervals over a period of six months and demonstrated the lability of the individual patient. Cander and Comroe,[12] reported daily variations of from 2 to 61 per cent in the vital capacity of asthmatic patients. Miller and co-workers point out that an individual patient may show considerable variation either when no treatment is given or in his response to treatment, emphasizing the need for critical evaluation of the bronchodilator effects of drugs administered to asthmatic patients.

In addition to inherent patient variability, spirometry is subject to limitations imposed by the instructions given the patient.[13] After a slow expiration, the vital capacity may be double the value obtained after a fast expiration, when forced expiration has collapsed the bronchi before maximal emptying of the lungs has been achieved. In patients with airway obstruction who are instructed to perform a submaximal expiration which results in a large vital capacity, there is prolongation of the terminal portion of the expirogram resulting in a decreased MMF. Conversely, when there is a decreased vital capacity without airway obstruction, there may be an attendant reduction of MMF and one-second vital capacity. It is seen, then, that improvement in measurements of airway obstruction may be small if there is an increase of vital capacity and that, in the presence of a large increase of vital capacity, an improvement in MMF acquires greater significance. It is of interest in this regard that Table III demonstrates a greater improvement of vital capacity among the emphysema patients than among the asthmatic patients, but a greater change of MMF in the asthma group. Part of the smaller change of MMF in the emphysema group may have resulted from the somewhat larger increase of vital capacity.

MMF and one-second vital capacity, which are both measurements of airway obstruction, are influenced by factors other than the size of the vital capacity. Franklin and associates[14] state that, paradoxically, in patients with

obstructive disease a maximal expiration yields a slightly slower rate of expiration than one that is submaximal. In the present study, the patients were directed to breathe out as rapidly and as deeply as possible, and the "best" breath was considered to be the one during which the largest volume of air was expired. Using this plan of patient instruction, a maximal rate of airflow may not have been achieved, but tests to be compared were performed in a standard fashion.

The placebo study, in addition to demonstrating the effectiveness of isoproterenol as a bronchodilator, attested to the occasional occurrence of wide variations in the immediate effect of a drug on an asthmatic patient. One patient showed an increase of MMF from 2.96 liters per second to 10.4 liters per second after administration of placebo. Subsequent ventilatory studies using the same placebo resulted in an insignificant change of MMF. This patient was unable to distinguish the subjective effects of placebo from those of isoproterenol. However, another patient with emphysema used placebo for twenty-four hours and was readily able to distinguish the "weak" placebo from isoproterenol and vehemently reported the difference when he returned breathless the next morning.

The subjective responses to the three forms of bronchodilator therapy compared in this study were also noted. Most patients preferred isoproterenol to the other two therapeutic programs, reporting a superiority in bronchodilator effect. Four patients found isoproterenol "too strong," disliked the taste, or complained that it "burned the tongue" despite proper use of the plastic adapter. One patient found that a piece of waxed paper placed over his tongue before his nightly inhalations eliminated this minor annoyance. With all forms of therapy, bronchodilator effects were noted immediately after inhalation of the aerosol and were reported to last only twenty to thirty minutes in a wheezing asthmatic patient or a severely dyspneic emphysema patient. More prolonged action was observed in the less severely distressed patients. It seems reasonable to advise the early use of aerosol bronchodilators before the development of a full-blown asthma attack or the occurrence of severe dyspnea.

During IPPB severely dyspneic or wheezing patients were occasionally unable to tolerate an initial pressure of 15 to 20 cm. of water. After treatment had been initiated with a pressure of 5 to 10 cm. of water a gradual increase to the desired level was possible.[3] There was no subjective preference for administration of the bronchodilator with IPPB. However, it has been shown that there is frequently a rather poor correlation between the subjective and objective evaluations of IPPB because of the marked psychologic effect of the imposing apparatus and procedure. This was apparent in those who preferred, as well as those who rejected, this form of therapy.[6] The findings reported in this study are in agreement with those of Miller and co-workers,[5] who found that there was no demonstrable advantage to the use of positive pressure with a bronchodilator over the use of the bronchodilator alone. They note that occasionally there may be considerable benefit from the use of positive pressure with bronchodilators when patients are incapable of voluntarily increasing their

tidal volumes, as in respiratory acidosis, abnormalities of the thoracic wall, and muscular deficiencies. However, most patients with emphysema who are ambulatory are capable of some voluntary increase in tidal volume.

There were no toxic effects resulting from the use of bronchodilator therapy in this group. Digilio and Munch[15] attested to the relative safety of these preparations and noted no appreciable change in blood pressure, pulse rate, electrocardiogram, or blood sugar levels after forty-eight successive inhalations of a bronchodilator in patients with cardiac disease, hyperthyroidism, or diabetes. Kaufman and associates[16] noted no change in cardiac output after administration of isoproterenol aerosols to patients. No detrimental effects were noted during this study following the use of IPPB in either asthma or emphysema patients. As pointed out by Motley and colleagues,[17] there is apparently no contraindication to the use of IPPB in severely emphysematous patients.

SUMMARY AND CONCLUSIONS

1. Spirometry was performed before and after the administration of a new form of isoproterenol aerosol (Medihaler-Iso) and placebo to eleven patients with bronchial asthma and fifteen patients with chronic pulmonary emphysema. Isoproterenol was found to have a bronchodilator effect which was independent of the alcohol-containing dispersing vehicle, the mode of nebulization, or the raising of secretions.

2. In a comparison of isoproterenol, epinephrine nebulized manually, and epinephrine nebulized by means of the Emerson intermittent positive pressure breathing valve, there were two probably significant results:

(a) Isoproterenol afforded a significantly greater improvement in one-second vital capacity than did epinephrine in emphysema patients.

(b) Isoproterenol improved the mean midexpiratory flow rate of patients with emphysema to a significantly greater degree than did epinephrine with positive pressure breathing.

3. There was no advantage to the administration of bronchodilators by intermittent positive pressure over the proper use of a hand nebulizer.

4. Patients with asthma attained a greater improvement in mean midexpiratory flow rate than did patients with emphysema with all three forms of bronchodilator therapy.

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COMPARING THE EFFECTIVENESS OF A PREDNISOLONE-HYDROXYZINE COMBINATION WITH PREDNISOLONE IN TREATMENT OF ALLERGIC DISEASES

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ANXIETY associated with allergic symptoms is of great concern to the patient as well as the doctor. Abramson[1] has clearly indicated the importance of psychogenic factors in allergic diseases, and it is clear that therapy of bronchial asthma, allergic rhinitis, allergic dermatoses, and other allergic disorders may not be entirely satisfactory in many patients unless specific measures are directed toward control of these psychogenic factors. Furthermore, while the corticosteroids have proved invaluable in the treatment of many patients unresponsive to other measures, their effect on the central nervous system may aggravate rather than allay symptoms of anxiety. In a previous study on the use of prednisone and prednisolone in allergy,[2] we reported that one-third of the side reactions involved the nervous system.

These considerations suggested to us that a tranquilizing drug might be useful, as an adjunct to corticosteroids, in allaying psychic disturbances causing or aggravating allergic attacks. We were also interested in determining whether a tranquilizing drug would reduce the incidence of steroid-induced effects on the nervous system.

Accordingly, a study was undertaken to evaluate the effectiveness of a corticosteroid administered in combination with a tranquilizer. Prednisolone, which has been demonstrated by many authors[3-6] to be effective, was selected as the steroid and was given in combination with hydroxyzine, a tranquilizing agent reported by Seneca,[7] Shalowitz,[8] Farah,[9] and Bayart[10] to be extremely successful in the management of anxiety states with a minimum of side reactions. Effective use of this combination in treating rheumatoid arthritis has recently been reported by Warter.[11]

PROCEDURE

The following design was employed so that the results achieved with several medications might be directly compared. Patients were treated for successive two-week intervals with each of four different medications. The order in which the medications were given in each case was randomly determined, and not all patients received all four medications. The four medications, with the prescribed daily dosage of each, were as follows: Prednisolone, 20 mg.;

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The prednisolone-hydroxyzine, prednisolone, and hydroxyzine used in this study were supplied as Ataraxoid, Sterane, and Atarax by the Medical Department of Pfizer Laboratories, a Division of Chas. Pfizer & Co., Inc., Brooklyn, New York.

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hydroxyzine, 40 mg.; prednisolone, 20 mg., together with hydroxyzine, 40 mg., and a placebo. All of the medications were administered as tablets of similar appearance, and were dispensed in bottles containing a week's supply. The medications were identified only by number, and the study was performed under double-blind conditions, so that neither the patients nor the investigators knew which medications were being used. Patients were seen and their conditions evaluated once a week throughout the study. Patients were provided with cards on which daily records of symptoms were kept; these records were considered in the final evaluation of each of the treatments as producing excellent, good, fair, or poor results.

The ninety-seven patients on whom the results of the study were based ranged in age from 9 to 76 years. Female patients outnumbered males approximately two to one. Table I shows the number of patients suffering from

TABLE I. ALLERGIC DISORDERS TREATED WITH FOUR DIFFERENT MEDICATIONS

ALLERGIC DISORDER	PREDNISOLONE-HYDROXYZINE COMBINATION	PREDNISOLONE	HYDROXYZINE	PLACEBO	TOTAL
Bronchial asthma	34	36	23	23	116
Vasomotor rhinitis	37	37	23	25	122
Allergic rhinitis	5	5	4	4	18
Urticaria	3	4	2	2	11
Neurodermatitis	4	3	1	2	10
Contact dermatitis	1	2	2	1	6
Conjunctivitis	2	2	1	1	6
Total	86	89	56	58	289

each disorder treated, as well as the number of trials of each compound. Of the ninety-seven patients, forty-three received all four drugs in succession, thirteen received three, thirty-seven received two, and four received only one.

In general, ancillary treatment was not altered during the course of the study. Hyposensitization was continued in those patients receiving it, and all patients were allowed to take any additional medication desired after duly recording the kind and amount on the diary card.

RESULTS

Most of the patients in this study suffered from bronchial asthma or vasomotor rhinitis. No significant differences between the two disorders were observed in the results of any of the drug trials. Therefore, all of the allergic disorders were considered together for the sake of simplicity in presentation.

The response of patients to each of the medications is shown in Table II. In several respects, significant differences were observed in the ability of the different medications to control allergic disorders. Excellent results were obtained with the prednisolone-hydroxyzine combination in a greater proportion of patients (75.6 per cent) than with prednisolone alone (48.3 per cent). This difference is highly significant ($P < 0.01$). Both the prednisolone-hydroxyzine combination and prednisolone alone produced a significantly greater proportion of excellent results than either of the other medications ($P < 0.05$).

TABLE II. RESPONSE OF ALLERGIC DISORDERS TO THERAPY

MEDICATION	NUMBER OF PATIENTS	RESPONSE (PER CENT)				SIDE REACTIONS (PER CENT)
		EXCELLENT	GOOD	FAIR	POOR	
Prednisolone-hydroxyzine combination	86	75.6	18.6	3.5	2.3	37.2
Prednisolone	89	48.3	40.5	5.6	5.6	37.2
Hydroxyzine	56	32.1	26.8	19.7	21.4	23.2
Placebo	58	24.1	10.3	19.0	46.6	15.5

The "excellent" and "good" categories may be grouped to show the proportion of patients in which the medications were of substantial benefit. When this was done, no significant difference was observed between the effectiveness of prednisolone (88.8 per cent) and that of the prednisolone-hydroxyzine combination (94.2 per cent). However, both were significantly more effective than the other medications ($P < 0.01$). Using this criterion of effectiveness we observed a highly significant difference ($P < 0.01$) between the effectiveness of hydroxyzine (58.9 per cent) and that of placebo (34.4 per cent). The results with placebo were consistent with results published elsewhere in treating a variety of disorders. Elimination of the so-called "placebo reactors" from the analysis did not materially alter the results of the study.

Side reactions were observed in 37 per cent of the patients when they were given prednisolone or the prednisolone-hydroxyzine combination, in 23 per cent when they were given hydroxyzine, and in 15 per cent when they were given placebo. Some of the patients had multiple side reactions. Table III lists all the reactions observed.

TABLE III. TOTAL NUMBER OF SIDE REACTIONS

SIDE REACTIONS	HYDROXYZINE-PREDNISOLONE	HYDROXYZINE	PREDNISOLONE	PLACEBO
Abdominal cramps	1	1	4	—
Overstimulation	3	—	5	3
Drowsiness	11	13	3	—
Nausea	—	—	5	1
Heartburn	4	1	7	1
Urinary frequency	4	—	5	—
Diarrhea	1	—	1	—
Headache	1	4	3	2
Increased appetite	8	—	6	—
Acneform rash	—	—	1	—
Nocturia	3	2	6	—
Constipation	2	—	2	—
Dryness of mouth	1	—	1	1
Edema	—	—	1	—
Palpitation	—	1	1	1
Dermatitis	1	—	—	—

DISCUSSION

It was possible, from these results, to rank the medications according to their effectiveness. The most successful of the four in the treatment of allergic disorders was the prednisolone-hydroxyzine combination, followed in order by prednisolone, hydroxyzine, and placebo. Eisenberg,[12] in discussing the role

of tranquilizing drugs in allergy, noted that sedative and relaxing effects produced a secondary result in reducing the intensity of allergic symptoms in a certain number of cases. We also feel this to be true, since the marked difference between patients on hydroxyzine-prednisolone and those on prednisolone alone was in the "excellent" category. It would be in the difference between "good" relief and "excellent" relief that we should expect to find the effectiveness of the secondary medication.

No effort was made in this study to determine whether a reduction of steroid dosage could be attained with the addition of the tranquilizer. One of us (E. B. B.) had the occasion to observe a patient, not included in this study, who had received 20 mg. of prednisolone daily for one year. Numerous attempts to lower the dosage resulted in a return of severe asthma. The patient was transferred to the hydroxyzine-prednisolone preparation and in three weeks it was possible to maintain her status on 10 mg. of prednisolone plus 10 mg. of hydroxyzine per day.

Side reactions occurred as frequently when the prednisolone-hydroxyzine combination was used as when prednisolone was used alone. It is interesting to note, however, that the incidence of drowsiness during the hydroxyzine trial, which occurred in 23 per cent of the patients, was cut almost in half when hydroxyzine was combined with prednisolone.

SUMMARY

1. A double-blind study has been conducted to compare the effectiveness in allergic diseases of therapy with prednisolone, hydroxyzine, a prednisolone-hydroxyzine combination, and a placebo.

2. The combination of prednisolone with hydroxyzine is significantly more effective than either prednisolone or hydroxyzine given separately.

3. Prednisolone, hydroxyzine, and the combination of both are all significantly more effective than placebo.

4. The incidence of drowsiness caused by hydroxyzine was reduced from 23 to 12 per cent when hydroxyzine was used in combination with prednisolone.

5. The incidence of side reactions did not differ in patients treated with prednisolone or with the prednisolone-hydroxyzine combination.

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Editorial

THE NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

INASMUCH as many members of the American Academy of Allergy are participating in the activities of the National Institute of Allergy and Infectious Disease, it seems appropriate to review at least a part of the program of that organization.

The most recent of the seven institutes that comprise the National Institutes of Health of the United States Public Health Service, the National Institute of Allergy and Infectious Disease has accomplished a great deal during the two years of its existence. Its interest in infectious diseases comprises bacteriology, virology, rickettsiology, mycology, and other related disciplines of public health, whereas allergy has been linked to immunology. There is an intramural program at the extensive medical center in Bethesda, Maryland, as well as an extramural program which consists largely of awarding grants for research, for which $10,824,000.00 was available last year.

Applications for research grants are referred to appropriate study sections comprised of authorities on the subjects involved. Three members of the Academy (Drs. Eisen, Sherman, Talmage) are on the Section of Allergy and Immunology. Dr. Sulzberger is on the general medicine study section. Recommendations of the study sections are sent to an Advisory Council for final action. One member of the academy (Dr. Alexander) is on the Council which deals with policies, new projects, and other matters in an advisory capacity to the Surgeon General.

As of Sept. 1, 1957, 162 grants, active or committed, totaling about $2,100,000.00 had been awarded to investigators whose problems concerned allergy and immunology. Over 60 per cent dealt with allergy, for which over $1,300,000.00 was awarded. Thirty-four research projects undertaken by thirty-one investigators who represent various classes of membership in the Academy received support to the extent of over $600,000.00. Still others were approved by the study section but, because of a lack of sufficient funds for all of the large number of applications received, only those above a given priority score could be paid.

A significant contribution to the interests of allergy has recently been effected. This new project concerns a training program which was established in the hope of attracting more qualified graduates to this specialty, as well as to others in which there is a dearth of such talent. The Council requested

special funds for this purpose and the project was approved by the various authorities concerned, including the Congress, which appropriated money for a substantial program.

For allergy, it was decided to set up patterns in which medical schools would serve as training centers. Such schools throughout the country were informed about available support for this program and many applications were received. An advisory committee consisting of persons well versed in medical education was then appointed to advise the Council in awarding the training grants. Various patterns of training were approved. In three medical schools (Johns Hopkins, New York University, and the University of Pittsburgh) sections of allergy will be established on a full-time basis in the departments of medicine. In these instances, allergy will have an appropriate place in the teaching curricula, so that medical students, house officers, and trainees will be exposed to good teaching, research, and clinical practice. It is hoped that the number of future trainees may thus be augmented. Through coordinated programs with other departments of the school in which there is interest in allergy a trainee will have the opportunity to become oriented to a broad aspect of hypersensitivity. In other schools (Cornell, University of Washington, University of Colorado, and California Institute of Technology) grants for training in pediatric allergy and for basic research have been awarded. Many applications are pending and further grants will be made to the limit of funds available ($240,000.00).* It is hoped that additional money will be appropriated so that the program may be expanded.

A third activity sponsored by the Institute concerns research fellowship awards which are given largely for basic investigation. Inasmuch as the number of applications pending far exceeds the funds available, the number of new awards is limited. However, two that concern allergy were given during the past year, although several others were continued.

Plans for other projects in the interest of allergy are in preparation and will be presented to the Advisory Council at forthcoming meetings.

*Since this report was written, training grants have been awarded to the medical schools of the University of Chicago, the University of Rochester, and several other institutions.

Announcements

Collegium Internationale Allergologicum Honors Dr. Béla Schick

New York, November 20.—At a dinner at the Hotel Pierre honoring Dr. Béla Schick, world-famous pediatrician, on his eightieth birthday, Dr. William Kaufman of Bridgeport, Connecticut, as a member of the governing council of the Collegium Internationale Allergologicum, conferred upon Dr. Schick the first honorary fellowship in this medical organization. The dinner which was sponsored by many national and international medical societies and other groups, was given under the auspices of the American Jewish Congress.

Although Dr. Schick is best known to the public for the Schick test, his method of testing for immunity to diphtheria, he has also done important research in allergy, scarlet fever, serum sickness, tuberculosis, and nutrition of the newborn and older children. Active at the age of 80, Dr. Schick is visiting professsor of pediatrics at the Albert Einstein College of Medicine and is affiliated with Mount Sinai Hospital, the Beth-El Hospital in Brooklyn, and the Bronx Municipal Hospital Center. He is presently doing research into the causes of infant prematurity.

The Collegium Internationale Allergologicum, founded in 1953, consists of eighty members from 18 different countries. Fellowship is by invitation only. No country may have more than twelve active fellows, and only those doctors and medical scientists who have made unusually important scientific contributions to allergy and allied fields may become fellows.

Among the present members of the Collegium are such men as Sir Henry H. Dale, winner of the 1936 Nobel Prize in Medicine; Professor Daniel Bovet, winner of the 1957 Nobel Prize in Medicine for his work with curare and the antihistaminics; Sir George W. Pickering, Regius Professor of Medicine at Oxford, who is noted for his work in the field of hypertension; Professor W. Löffler, who discovered a lung ailment called Löffler's syndrome; Dr. R. R. A. Coombs, who developed the diagnostic Coombs test for identifying certain antibodies which are capable of causing disease; Dr. Carl Prausnitz, co-discoverer of the Prausnitz-Küstner passive-transfer test which permits the demonstration of local hypersensitiveness in man by the injection of the serum of an allergic person into the skin of a normal person; and Professor O. Ouchterlony, who devised the Ouchterlony plate which is an important tool in antigen-antibody analysis.

The nine American fellows of the Collegium are Drs. E. A. Kabat, M. Murray Peshkin, and Harold G. Wolff of New York; F. F. Yonkman and R. L. Mayer of Summit, New Jersey; Sidney Raffel of Stanford, California; George L. Waldbott of Detroit, Michigan; Roger P. Wodehouse of Pearl River, New York; and William Kaufman of Bridgeport, Connecticut.

A festschrift or birthday volume to honor Dr. Schick on the occasion of his eightieth birthday is being prepared for publication in the spring as a special issue of the *International Archives of Allergy and Applied Immunology*. Leading medical teachers, scientists, and physicians from the United States and foreign countries are contributing major scientific papers on various aspects of allergy and immunology. Dr. William Kaufman will edit this volume, which will be published by S. Karger of Basel, Switzerland, and New York, N. Y.

At the dinner honoring Dr. Schick, Dr. Rene Dubos of the Rockefeller Institute for Medical Research presented Dr. Schick with the Annual Award for Service to Mankind given by the Business and Professional Associates of the American Jewish Congress. Previous winners of this award were Dr. Selman A. Waksman, Dr. Robert M. Hutchins, and Brigadier General Morris C. Troper.

New York Allergy Society

The New York Allergy Society has elected the following officers for 1958:

President, Leoni N. Claman
President-Elect, Aaron D. Spielman
Vice-President, Louis Sternberg
Secretary, Sheppard Siegal
Treasurer, Joseph H. Fries
Assistant Secretary-Treasurer, S. Senior Sack.

Philadelphia Allergy Society

The officers of the Philadelphia Allergy Society for 1958 are as follows:

President, Horace Petit
Vice-President, Alexander Peters
Secretary-Treasurer, Eugene A. Hildreth.

Residencies Available in Pittsburgh

Applications are being received for appointment to a one-year approved residency in allergy at the Montefiore and Medical Center Hospitals as well as at the Veterans Administration Hospital in Pittsburgh, Pennsylvania.

Please address inquiries to Leo H. Criep, M.D., Bigelow Apartments, Pittsburgh 19, Pennsylvania.

Florida Allergy Society

The new officers of the Florida Allergy Society are as follows:

President, Norris M. Beasley
Vice-President and President-Elect, George F. Hieber
Secretary-Treasurer, I. Irving Weintraub.

Book Review

Psychosomatic Medicine: A Clinical Study of Psychophysiologic Reactions. By E. Weiss and O. S. English. Third edition. Philadelphia, 1957, W. B. Saunders Company. 557 pages. Price, $10.50.

This is the third edition of what has become a standard textbook in the special field of psychosomatic medicine. The authors, respectively professor of clinical medicine and professor of psychiatry at Temple University Medical Center, have kept abreast of the voluminous writing in this area. Work here has been largely clinical, as their text aims to be. The devotee of strict scientific demonstration, whether he is a student of psychic or of bodily processes, will find points to criticize. The psychosomatic approach as a discipline is still relatively new and often overenthusiastic, relying more on anecdote and conviction than painstaking experiment. But the reader will also be impressed by the sheer weight of clinical evidence adduced and the beginnings made by such workers as Wolf and Wolff, Margolin, or Mirsky and his associates at introducing precise experimental methods into this complex field.

The critical allergist may be more disappointed than some other specialists. Advances have not been so striking in his discipline. The authors present what evidence there is concerning the role of emotional factors in allergic conditions, taking care to include opposing points of view, such as the extraordinary attitude of Sulzberger that emotions have nothing whatsoever to do with the pathogenesis of skin disorders. Drs. Weiss and English have a frank bias in the opposite direction. They believe that their approach is here to stay but, above all, they stress the dangers of adherence to a rigid all-or-none, "either-or" viewpoint.

The clinical allergist who wishes to browse beyond the strict confines of wheal and wheeze will find in the early chapters of the book a simple account of personality development and of how psychodynamic principles may be applied in the diagnosis and management of patients.

Peter H. Knapp

Obituaries

BRET RATNER
1893-1957

BRET RATNER died on Oct. 11, 1957, of a coronary occlusion. Fellow members of the American Academy of Allergy, as well as all others who are interested in the field of allergy, have suffered a great loss in his passing.

Dr. Ratner received his doctor of medicine degree from the College of Medicine of New York University in 1918, following which he was an intern and assistant pathologist at City Hospital and later an intern and resident in pediatrics at the New York Nursery and Child's Hospital. During these years, under the influence of the late Oscar M. Schloss, Dr. Ratner became interested in pediatric allergy and in 1921 entered into private practice in New York City, specializing in this field. At the same time he began to carry on research in allergy and immunology under the late Hans Zinsser at Columbia College of Physicians and Surgeons and later at the College of Medicine of New York University.

His studies encompassed investigations in the role of colostrum, placental permeability to antigens and antibodies, and experimental asthma in guinea pigs. Among his many significant contributions during his early years in research were the demonstration of successful sensitization and anaphylactic shock of guinea pigs by the nasal route and later the demonstration of the production of sensitization and asthma in the guinea pig by inhalation of dried antigenic powders.

In 1926 and 1927 he published a classic series of studies with Holmes C. Jackson and Helen Lee Gruehl in which he reported successful active and passive sensitization of the guinea pig fetus in utero and at the same time published further studies of the role of colostrum and milk in sensitizations during the neonatal period. This, together with a clinical study in 1932, led him to believe that infants could be actively sensitized in utero to various foods eaten excessively by mothers during the last trimester of pregnancy. This was followed by studies on the anaphylactogenic properties of raw and modified foodstuffs. These studies continued until his death; several of them are being completed posthumously by his co-workers.

In 1943 he was appointed director of pediatrics at the Seaview Hospital for Tuberculosis, Staten Island, New York, a post he held until his death. Among the investigations carried out during this period were those on the care and fate of infants born of tuberculous mothers and the use of isoniazid therapy in childhood tuberculous meningitis.

In 1949 Dr. Ratner resigned from the New York University College of Medicine, after having served twenty-two years as clinical professor of pediatrics, to accept an appointment as professor of clinical pediatrics and associate professor of immunology at the New York Medical College and attending pediatrician and director of pediatric allergy at the Flower and Fifth Avenue Hospitals. Among other studies during this period was one with Cecil Collins-Williams and Samuel Untracht in which he advanced a concept of the "Allergic Dermal-Respiratory Syndrome in Children."

Dr. Ratner was well known to all as a dynamic, forceful, outspoken man with the courage to express his true opinions, even when those opinions were unpopular. He dissented from the opinion that allergic disease is hereditary, and he also disagreed with those who considered histamine, acetylcholine, or other circulating substances as being of more than minor importance in the basic mechanism of the allergic reaction.

In addition to scientific papers, he contributed chapters to many books and was author of the book entitled *Allergy, Anaphylaxis, and Immunotherapy* (Baltimore, 1943, Williams & Wilkins Company).

His work was by no means confined to clinical and laboratory research, for he was also a practicing physician in the finest traditions of the profession. He always considered himself primarily a pediatrician, and he believed that allergy could be practiced properly only when it was considered as a subspecialty of the major field. His approach to his young patients was most conscientiously comprehensive and, although he objected to what he considered the overemphasis of psychogenic factors in allergic disease, he was friend and adviser as well as physician to his child patients and their parents.

Dr. Ratner was a member of the American Association of Immunologists, the Society for Experimental Biology and Medicine, and the American Society of Experimental Pathology. He was a Fellow of the American Academy of Pediatrics and the American College of Chest Physicians. He was also active in a number of local and honorary societies, including Alpha Omega Alpha and Sigma Xi. He was a Fellow of the New York Academy of Sciences. He was a founder and first chairman of the Allergy Section of the American Academy of Pediatrics, a member of the Board of Regents of the American College of Allergists, and vice-chairman of the Sub-Board of Pediatric Allergy of the American Board of Pediatrics.

Dr. Ratner was affiliated with the editorial staffs of a number of scientific publications, including the *International Archives of Allergy and Applied Immunology,* the *Quarterly Review of Allergy and Applied Immunology,* the *Annals of Allergy, Folia Clinica Internacional,* and the *Seaview Hospital Bulletin.*

Dr. Ratner was held in high affection by his students, both the undergraduates with whom he was in contact since his early days and by the members of the postgraduate course which he conducted at the New York Medical College. His postgraduate students have formed a Bret Ratner Club, which has met for several years at annual meetings of the American Academy of Pediatrics

and the American College of Allergists. He always gave unhesitatingly of his time and effort to his colleagues and to younger men who came to him for advice and guidance.

Dr. Ratner's death is a great loss, not only to his family, to the field of allergy, and to countless colleagues and friends, but also to the children for whom he did so much.

He is survived by his wife, Jeanne; three brothers, George and Victor of New York City and Herbert, Commissioner of Health of Oak Park, Illinois; a daughter, Mrs. Barbara Dworetzky of New York City; and two grandchildren, Tommy and Jody.

M. D.

MAX HARTEN
1906-1957

MAX HARTEN was born in New York City on April 9, 1906, and died on May 24, 1957, of carcinoma of the colon. He was graduated from the Long Island College of Medicine in 1934. He interned at the Jewish Hospital of Brooklyn from 1934 to 1937 and served as resident-in-allergy there.

Dr. Harten was adjunct attending allergist at Jewish Hospital of Brooklyn until 1953. He was associate editor of *Allergy Abstracts* from 1937 to 1953. At the time of his death, he was a member of the staffs of the Kings County Hospital and the North Shore Hospital. Dr. Harten was a Diplomate of the Board of Internal Medicine and Allergy and a Fellow of the American Medical Association, the American Academy of Allergy, the American College of Physicians, and the New York Allergy Society. He wrote many articles on allergy.

Dr. Harten is survived by his widow, Dorothy Harten, and a daughter, Elizabeth.

His death is a great loss to his colleagues and friends, his patients, and his family.

M. M. A.